

M.H

13/01

PCT / CA 99 / 00600
22 OCTOBER 1999 (22.10.99)

IA 122567

REC'D 10 NOV 1999

WIPO PCT

EAJU

THE UNITED STATES OF AMERICA

TO ALL TO WHOM THESE PRESENTS SHALL COME:
UNITED STATES DEPARTMENT OF COMMERCE

United States Patent and Trademark Office

July 13, 1999

THIS IS TO CERTIFY THAT ANNEXED HERETO IS A TRUE COPY FROM
THE RECORDS OF THE UNITED STATES PATENT AND TRADEMARK
OFFICE OF THOSE PAPERS OF THE BELOW IDENTIFIED PATENT
APPLICATION THAT MET THE REQUIREMENTS TO BE GRANTED A
FILING DATE UNDER 35 USC 111.

APPLICATION NUMBER: 60/091,683

FILING DATE: July 02, 1998

PRIORITY DOCUMENT

SUBMITTED OR TRANSMITTED IN
COMPLIANCE WITH RULE 17.1(a) OR (b)



By Authority of the
COMMISSIONER OF PATENTS AND TRADEMARKS

T. Wallace
T. WALLACE
Certifying Officer

PROVISIONAL APPLICATION FOR PATENT COVER SHEET

This is a request for filing a PROVISIONAL APPLICATION under 37 CFR 1.53(c).

jc542 U.S. PTO
60/091683

07/02/98

jc408 U.S. PTO
07/02/98

DOCKET NUMBER		1960.86 PV		Type a plus sign (+) inside this box.	+
INVENTOR(s)/APPLICANT(s)					
LAST NAME	FIRST NAME	MIDDLE INITIAL	RESIDENCE (CITY AND EITHER STATE OR FOREIGN COUNTRY)		
1. PON	1. RICHARD	1. T.	1. 108 Ranch Estates Road N.W. Calgary, Alberta, Canada T3G 2B4		
2. YU	2. SHUYUAN	2. N/A	2. 53 Hawktree Circle N.W. Calgary, Alberta, Canada T3G 3M1		
TITLE OF THE INVENTION (280 characters max)					
REUSABLE SOLID SUPPORT FOR OLIGONUCLEOTIDE SYNTHESIS PROCESS FOR PRODUCTION THEREOF AND PROCESS FOR USE THEREOF					
CORRESPONDENCE ADDRESS					
FITZPATRICK, CELLA, HARPER & SCINTO 277 Park Avenue New York, N.Y. 10172 Telephone No. (212) 758-2400					
ENCLOSED APPLICATION PARTS (check all that apply)					
<input checked="" type="checkbox"/> Specification Number of Pages <u>85</u> <input type="checkbox"/> Small Entity Statements					
<input checked="" type="checkbox"/> Drawings Number of Sheets <u>3</u> <input type="checkbox"/> Other (specify) _____					
METHOD OF PAYMENT (check one)					
<input type="checkbox"/> A check or money order is enclosed to cover the Provisional filing fees					PROVISIONAL FILING FEE AMOUNT (\$)
<input checked="" type="checkbox"/> The Commissioner is hereby authorized to charge filing fees and credit Deposit Account Number: <u>06-1205</u>					
					\$150.00

07/02/98

The invention was made by an agency of the United States Government under a contract with an agency of the United States Government.

☒ No.

☐ Yes, the name of the U.S. Government agency and the Government contract number are: _____

Respectfully submitted,

SIGNATURE

Richard P. Bauer

DATE

July 2, 1998

TYPED or PRINTED NAME

RICHARD P. BAUER

REGISTRATION NO.

31,588

(if appropriate)

☐ Additional inventors are being named on separately numbered sheets attached hereto.

PROVISIONAL APPLICATION FOR PATENT FILING ONLY

F501\W161327\dew

FINAL VERSION

Applicants/Inventors: Richard T. Pon
Shuyuan Yu

Title: Reusable Solid Support For Oligonucleotide Synthesis,
Process For Production Thereof and Process For Use Thereof

Assignee: University Technologies International Inc.

JURISDICTION: United States Provisional

DATE: July 2, 1998

IP Doc #: 98436-1

BACKGROUND OF THE INVENTION

FIELD OF THE INVENTION

In one of its aspects, the present invention relates to a reusable solid support for oligonucleotide synthesis. In another of its aspects, the present invention relates to a process for production of such a reusable solid support. In yet another of its aspects, the present invention relates to a process for use of such a reusable solid support.

DESCRIPTION OF THE PRIOR ART

The art of organic chemistry on solid supports is generally known. A useful review article on this topic may be found in "Organic Chemistry on Solid Supports" by Früchtel et al., *Angew. Chem. Int. Ed. Engl.*, 1996, 35, pgs. 17-42, the contents of which are hereby incorporated by reference.

As discussed in Früchtel et al., the art has developed automated solid-phase synthesis of polypeptides, oligonucleotides and oligosaccharides. Of particular interest here is solid-phase synthesis of oligonucleotides. The following are useful review articles/textbooks on this topic:

Beaucage et al., *Tetrahedron*, 1992, 48, 2223;

Davis et al., *Innovation and Perspectives in Solid Phase Synthesis* (Ed.:

R. Epton), Intercept, Andover, 1992, pg. 63;

Montserra et al., *Tetrahedron*, 1994, 50, 2617; and

S. L. Beaucage et al., *Tetrahedron*, 1993, 49, 6123-6194;

the contents of each of which are hereby incorporated by reference.

In the solid-phase synthesis of oligonucleotides, it is known to synthesize the oligonucleotide on an inorganic solid support bearing a succinyl linker arm - see, for example, any of the following references:

Caruthers et al., *Genetic Engineering*, Plenum Press, New York (1982),

Vol. 4, pgs. 1-17;

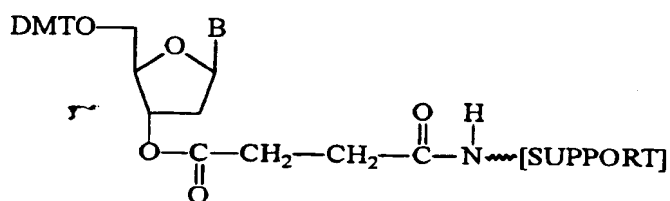
Letsinger et al., *Genetic Engineering*, Plenum Press, New York (1985),
Vol. 5, pg. 191;

Froehler et al., *Nucleic Acids Research*, 14:5399-5407 (1986); and

Matteucci et al., *Journal of American Chemical Society*, 103:3185-
3186 (1981);

the contents of each of which are hereby incorporated by reference.

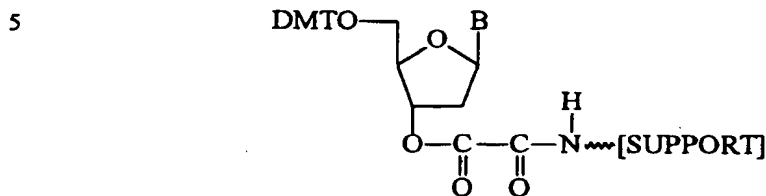
Typically, the succinyl linker arm has the following general formula:



Thus, the succinyl group links the growing oligonucleotide from its terminal 3' hydroxyl group by an ester bond to a primary amine on the support, which may be, for example, conventional controlled pore glass (CPG) or silica, by an amide bond. Once the desired oligonucleotide has been synthesized, it is freed or cleaved from the succinyl linker arm hydrolyzing the ester carbonyl group. The hydrolysis agent is usually concentrated ammonium hydroxide. Typically, this reaction can take from 1-4 hours to complete. With improvements to current solid-phase oligonucleotide synthesizers, this cleavage step can represent 50% or more of the total time require to synthesize the desired oligonucleotide.

Another type of linker arm is disclosed in United States patent 5,112,962 [Letsinger et al. (Letsinger)], the contents of which are hereby incorporated by reference.

Letsinger teaches a linker arm for solid support synthesis of oligonucleotides and oligonucleotide derivatives have the following formula:



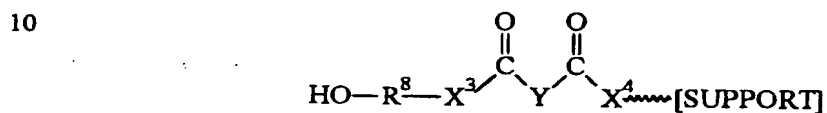
10

Thus, Letsinger teaches an oxalyl linker arm which purportedly release the synthesized oligonucleotide or oligonucleotide derivate in a period of 1-30 minutes in a manner that leaves the oligonucleotide fully protected. The oxalyl linker arm purportedly can be rapidly cleaved by 5% ammonium hydroxide in methanol, ammonium hydroxide, wet tertiary amine, triethylamine/alcohol, triethylamine/methanol, triethylamine/ethanol, aqueous trimethylamine and other bases. Unfortunately, the oxalyl linker arm of Letsinger suffers from its purported advantage. Specifically, the present inventors have discovered that the oxalyl linker arm of Letsinger is susceptible to significant spontaneous hydrolysis (e.g. spontaneous hydrolysis of ~10-40% per month) which renders it difficult to use in commercial operations. The oxalyl arm is also difficult to prepare because it requires using oxalyl chloride, which is highly reactive, toxic and therefore dangerous.

Regardless of the specific nature of the linker arm, it is generally accepted in the art that the linker arm is not reusable after production and cleavage of the desired oligonucleotide. Thus, conventional linker arms may be regarded as non-recyclable. This is illustrated in Figure 1 which illustrates the conventional use of a succinyl linker arm for the production of an oligonucleotide. Thus, as illustrated, after cleavage of the desired oligonucleotide, the support is irreversibly linked to the linker compound (i.e., the succinyl moiety) and cannot be reused.

The art is in need of a linker arm for solid support oligonucleotide synthesis, which linker arm is recyclable. More specifically, the art is in need of a linker arm capable of repeated oligonucleotide synthesis/cleavage.

In published International patent application WO 97/23496 [Pon et al.], the contents of which are hereby incorporated by reference, there is reported the first recyclable linker arm. This linker arm is based on a derivatized solid support having the following formula:

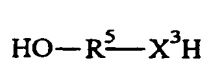


wherein: R^8 is selected from the group consisting of a substituted or unsubstituted C_1 - C_{20} alkyl group, a substituted or unsubstituted C_3 - C_{30} aryl group and a substituted or unsubstituted C_3 - C_{40} alkylaryl group; X^3 and X^4 are the same or different and are selected from the group consisting of -O-, -S-, -S(O)₂- and -N(R^{12})-; R^{12} is selected from the group consisting of a substituted or unsubstituted C_1 - C_{20} alkyl group, a substituted or unsubstituted C_3 - C_{30} aryl group and a substituted or unsubstituted C_3 - C_{40} alkylaryl group; and Y is selected from the group consisting of:

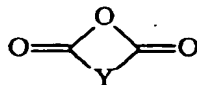
-CH ₂ -CH ₂ -;	-CH ₂ -;
-CH ₂ -O-CH ₂ -;	-CH ₂ -CH ₂ -CH ₂ -;
-CH=CH-;	-CH=C(CH ₃)-;
-C(CH ₃)=C(CH ₃)-;	-CH ₂ -C(=CH ₂)-; and
-CH ₂ -S-CH ₂ -.	

While a linker arm based on the solid support described by Pon et al. is a significant advance in the art, there is still room for improvement. Specifically, the solid support described by Pon et al. has the following disadvantages.

First, prior to attachment of the linker moiety, the solid support must be derivatized by a process comprising the step of reacting together the compounds of Formulae I, II and III:



(I)



(II)



(III)

wherein R^5 , X^3 , X^4 and Y are as defined above. Practically, this involves two steps - i.e., reaction of the compound of Formula III with one of the compounds of Formulae I and II and subsequent reaction with the other of compounds of Formulae I and II. Thus, the disadvantage is additional labour required to effect a two-step derivatization of the solid support.

Second, each step of the derivatization described in the previous paragraph has the potential of incompletely derivatizing each HX^4 - moiety on the support thereby increasing the likelihood of a heterogeneous surface. Practically, it becomes necessary to block or cap underivatized HX^4 - moieties so that the linker moiety does interact with them. Thus, the disadvantage is additional labour and cost required to effect derivatization of the solid support.

Third, a linker arm based on the derivatized support described by Pon et al. is not as resistant to partial cleavage during regeneration as a derivatized support having a more fully saturated moiety.

In light of these disadvantages, it would be desirable to have an improved recyclable solid state support material useful in the oligonucleotide synthesis. It would

be especially desirable if the the linker moiety could be attached to the support material with little or no derivatization required of the latter.

SUMMARY OF THE INVENTION

5 It is an object of the present invention to provide a novel solid support for oligonucleotide synthesis which obviates or mitigates at least one of the above-mentioned disadvantages of the prior art.

It is another object of the present invention to provide a novel process for producing the solid support.

10 It is an object of the present invention provide a novel linker arm for solid support oligonucleotide synthesis which obviates or mitigates at least one of the above-mentioned disadvantages of the prior art.

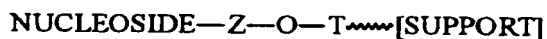
It is another object of the present invention to provide a novel process for producing a linker arm for solid support oligonucleotide synthesis.

15 Accordingly, in one of its aspects, the present invention provides a reusable linker arm for solid support oligonucleotide synthesis, the linker arm comprising the following formula:



wherein Z is a linker moiety and T is an organic radical.

25 In another of its aspects, the present invention provides a reusable linker arm for solid support oligonucleotide synthesis, the linker arm comprising the following formula:



30

wherein Z is a linker moiety and T is an organic radical.

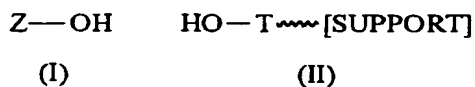
In yet another of its aspects, the present invention provides a process for production of a reusable linker arm for oligonucleotide synthesis having the following formula:

5



10 wherein Z is a linker moiety and T is an organic radical, the process comprising the step of reacting together the compounds of Formulae I and II:

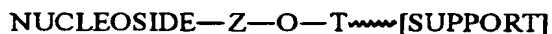
15



wherein Z and T are as defined above.

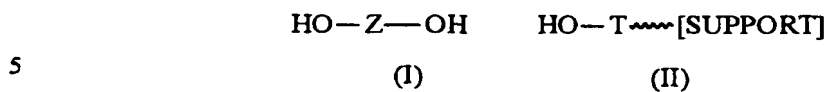
20 In another of its aspects, the present invention provides a process for production of a reusable linker arm for oligonucleotide synthesis having the following formula:

25



wherein Z is a linker moiety and T is an organic radical, the process comprising the step of reacting together the compound of Formulae I, II and III:

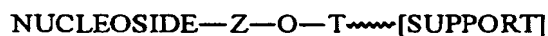
30



wherein Z and T are as defined above.

In yet another of its aspects, the present invention provides a process for producing an oligonucleotide having a desired sequence comprising the steps of:

- 15 (i) reacting a linker arm having the formula:



20 wherein Z is a linker moiety and T is an organic radical, with at least one oligonucleoside base until an oligonucleotide having the desired sequence is produced;

(ii) cleaving the oligonucleotide having the desired sequence to produce a free oligonucleotide having the desired sequence; and a used linker arm; and

- 25 (iii) recycling the used linker arm to Step (i).

As used throughout this specification, the term "oligonucleotide" is intended to have a broad meaning and encompasses conventional oligonucleotides, backbone-modified oligonucleotides (e.g. phosphorothioate, phosphorodithioate and methylphosphonate analogs useful as oligotherapeutic agents) and oligonucleotide derivatives
30 such as oligonucleotide-peptide conjugates.

Throughout this specification, when reference is made to a substituted moiety, the nature of the substitution is not specification restricted and may be selected from the group consisting of a C₁-C₂₀ alkyl groups, a C₃-C₃₀ aryl group a C₃-C₄₀ alkaryl group.

5

BRIEF DESCRIPTION OF THE DRAWINGS

Embodiments of the present invention will be described with reference to the accompany drawing in which:

Figure 1 illustrates a specific process pathway for conventional oligonucleotide synthesis; and

10

Figure 2 illustrates a specific preferred embodiment of the present invention.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

Initially, to facilitate an understanding of the invention, reference will be made to Figure 1, which illustrates a conventional process for solid support oligonucleotide synthesis.

15

Thus, the initial step of the process illustrated in Figure 1 comprises reacting a linking compound, such as succinic acid (while succinic acid is illustrated, succinic anhydride may also be used), with a conventional amine-terminated support. The reaction results in the formation of an amide linkage between the linking compound and the support to produce succinyl-support conjugate.

20

Next, the succinyl-support conjugate is reacted with a desired initial nucleoside to produce a linker arm. In the illustrated nucleoside, DMT is dimethoxytrityl, B is the nucleobase and R' is H (for deoxyribonucleosides) or OR (for ribonucleosides) wherein R is H or a conventional blocking/ protecting group. The reaction results in the formation of an ester linkage between the linking compound and the desired initial nucleoside at the 3' position of the latter.

25

The linker arm is then used in conventional oligonucleotide synthesis (e.g. in a conventional automated synthesizer) to produce an oligonucleotide of desired sequence attached to the linker arm.

The oligonucleotide is then cleaved from the linker by hydrolysis. This serves to cleave the ester bond thereby freeing the oligonucleotide and an amine-terminated, non-reusable linker arm.

5 The present inventors have surprisingly and unexpectedly discovered that a support having a hydroxy-terminated functionality may be combined with a conventional linking compound to produce linker arm which may used to synthesize an oligonucleotide of desired sequence. A key feature of the invention is that the linker arm may be regenerated or recycled after cleavage of the oligonucleotide of desired sequence. To the inventors' knowledge, this is the first discovery of a derivatized support which may be
10 used repeatedly in oligonucleotide synthesis.

The reusable linker arm of the present invention has the following formula:



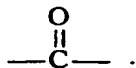
wherein Z is a linker moiety and T is an organic radical.

Preferably, T contains at least one carbon.

20 Preferably, T is a C₁-C₃₀₀ organic moiety, more preferably a C₁-C₂₀₀ organic moiety, most preferably a C₁-C₁₀₀ organic moiety.

As will be appreciated by those of skill in the art, T may be a saturated or unsaturated organic moiety. Further, T may contain one or more heteroatoms. For example, T may comprise at least one heteroatom selected from N and O.

25 In one preferred embodiment, the organic moiety in T comprises at least one moiety having the formula:



5

In another preferred embodiment, the organic moiety in T comprises at least one moiety having the formula:

10



In yet another preferred embodiment, the organic moiety in T comprises at least one moiety having the formula:

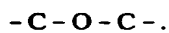
15



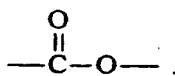
20

In yet another preferred embodiment, the organic moiety in T comprises at least one moiety having the formula:

25



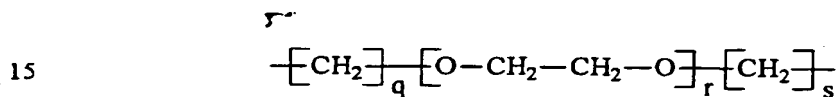
In yet another preferred embodiment, the organic moiety in T comprises at least one moiety having the formula:



5

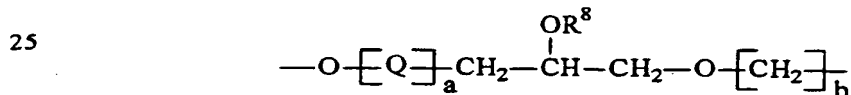
Further, those of skill in the art will recognize that the organic moiety in T may be unsubstituted or substituted. For examples, the organic moiety of T may be substituted by at least one moiety selected from the group comprising a C₁-C₄₀ alkyl group, a C₃-C₄₀ aryl group, a C₁-C₄₀ alkoxy group, a C₁-C₄₀ ester group, a C₁-C₄₀ hydroxy group, a C₂-C₄₀ acrylate group and a C₃-C₄₀ alkylaryl group.

In one preferred embodiment, T has the formula:



wherein q and s are the same or different and each is an integer having a value of 0-40 and r is an integer having a value of 1-200. In this embodiment, it is further preferred that q and s are the same or different and each is an integer having a value of 1-20 and r is an integer having a value of 1-150.

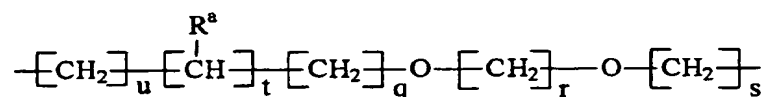
In another preferred embodiment, T has the formula:



wherein a is 0 or 1, Q is an organic moiety, R⁸ is hydrogen or a protecting group and b is an integer having a value of 0-40. In this embodiment, a may be 0 and R⁸ may be

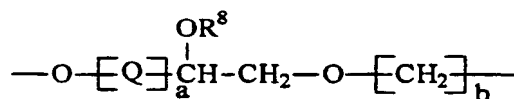
hydrogen. Further, a may be 1 and R^a may be a protecting group. Non-limiting examples of protecting groups may be selected from the group comprising acetyl, chloroacetyl, methoxyacetyl, *i*-butyl phenoxyacetyl, phenoxyacetyl, trityl, methoxytrityl, dimethoxytrityl (DMT), dialkylphosphite, pivalyl-isobutyloxycarbonyl, *t*-butyldimethylsilyl, phenoxyacetal, 9-phenylxanthen-9-yl (pixyl), tetrahydropyranyl, methoxytetrahydropyranyl, methoxymethyl, benzyloxymethyl, methoxyethoxymethyl, methylthiomethyl, dialkylphosphate, levuliny, dimethylphenylsilyl, trimethylsilyl, isopropyldimethylsilyl, diisopropylmethylsilyl, diethylisopropylsilyl, triisopropylsilyl, acetyl, benzoyl, pivaloyl, trifluoroacetyl, allyl, benzyl, *o*-nitrobenzyl, *o*-hydroxystyryldimethylsilyl, 2-oxo-1,2-diphenylethyl, allyloxycarbonyl, monomethoxymethyl, nitroveratryloxycarbonyl, dimethoxybenzoin, dimethoxybenzoin carbonate, methylnitropiperonyl carbonate, fluorenylmethoxycarbonyl, 2-phenylsulfonyl-ethoxycarbonyl, fluorophenyl-methoxypiperidinyl and mixtures thereof.

In this embodiment, Q may be a moiety having the formula:



wherein q, r, s, t and u are the same or different and each is an integer having a value of 0-40 and R^a is selected from the group comprising hydrogen, hydroxyl, a C₁-C₄₀ alkyl group, a C₃-C₄₀ aryl group, a C₁-C₄₀ alkoxy group, a C₁-C₄₀ ester group, a C₁-C₄₀ hydroxy group, a C₂-C₄₀ acrylate group and a C₃-C₄₀ alkylaryl group. Preferably, s is 0, q, r and u are the same or different and each is an integer having a value of 1-10, t is an integer of 1-5 and R^a is hydroxyl.

In yet another preferred embodiment, T has the formula:

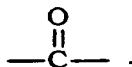


5

wherein a is 0 or 1, Q is an organic moiety, R⁸ is selected from the group comprising hydrogen, hydroxyl, a C₁-C₄₀ alkyl group, a C₃-C₄₀ aryl group, a C₁-C₄₀ alkoxy group, a C₁-C₄₀ ester group, a C₁-C₄₀ hydroxy group, a C₂-C₄₀ acrylate group and a C₃-C₄₀ alkylaryl group, and b is an integer having a value of 0-40. Preferably, Q is a C₁-C₁₀₀ organic moiety. As will be appreciated by those of skill in the art, Q may be a saturated organic moiety or an unsaturated organic moiety.

It is preferred that Q is a C₁-C₁₀₀ organic moiety comprising at least one heteroatom selected from N and O.

15 In one preferred embodiment, the organic moiety Q comprises at least one moiety having the formula:



20

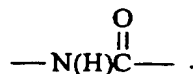
In another preferred embodiment, the organic moiety Q comprises at least one moiety having the formula:

25



In yet another embodiment, the organic moiety Q comprises at least one moiety having the formula:

30



5

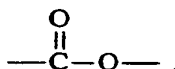
In yet another embodiment, the organic moiety Q comprises at least one moiety having the formula:

10



In yet another embodiment, the organic moiety comprises at least one moiety having the formula:

15

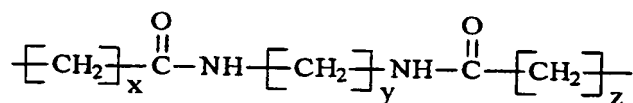


20

As will be appreciated by those of skill in art, the organic moiety Q may unsubstituted or substituted. For example, the organic moiety Q may be substituted by at least one moiety selected from the group comprising a C₁-C₄₀ alkyl group, a C₃-C₄₀ aryl group, a C₁-C₄₀ alkoxy group, a C₁-C₄₀ ester group, a C₁-C₄₀ hydroxy group, a C₂-C₄₀ acrylate group and a C₃-C₄₀ alkylaryl group.

25

In one preferred embodiment, Q has the formula:

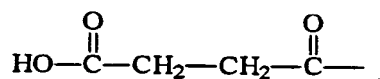


wherein each of x, y and z is an integer having a value of 1-40.

In the above formula for the present linker arm, Z is a linker moiety. As will be discussed below, Z is derived from a linker compound have the general formula HO-Z-OH (Formula I below). The nature of the linker compound is not particularly restricted.

5 In one preferred embodiment, linker moiety Z has the formula:

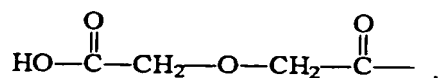
7



10

As will be apparent to those of skill in the art, this linker moiety may be derived from succinic acid or succinic anhydride.

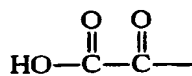
15 In another preferred embodiment, linker moiety Z has the following formula:



20

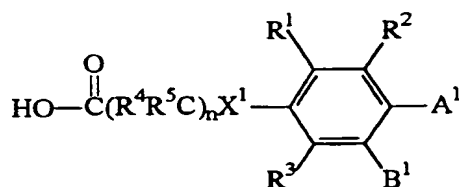
As will be apparent to those of skill in the art, this linker moiety may be derived from diglycolic acid or diglycolic anhydride.

In yet another preferred embodiment, linker moiety Z has the following formula:



10 As will be apparent to those of skill in the art, this linker moiety may be derived from oxalic acid or oxalyl chloride.

In yet another, and most, preferred embodiment, linker moiety Z has the following formula:

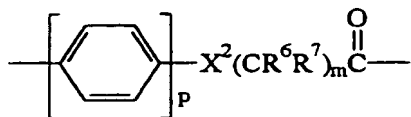


wherein: R^1 , R^2 and R^3 are the same or different and are selected from the group consisting of hydrogen, halide, a substituted or unsubstituted C_1 - C_{20} alkyl group, a substituted or unsubstituted C_3 - C_{30} aryl group and a substituted or unsubstituted C_3 - C_{40} alkylaryl group; R^4 and R^5 are the same or different and are selected from the group consisting of hydrogen, a substituted or unsubstituted C_1 - C_{20} alkyl group, a substituted or unsubstituted C_3 - C_{30} aryl group and a substituted or unsubstituted C_3 - C_{40} alkylaryl group; X^1 is selected from the group consisting of $-\text{O}-$, $-\text{C}(\text{O})-$, $-\text{S}-$, $-\text{S}(\text{O})_2-$ and $-\text{N}(\text{R})-$; R is selected hydrogen, a substituted or unsubstituted C_1 - C_{20} alkyl group, a substituted or

25

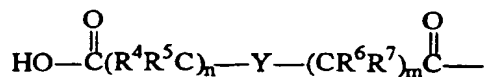
30

unsubstituted C₃-C₃₀ aryl group and a substituted or unsubstituted C₃-C₄₀ alkylaryl group; n is 0, 1 or 2; and one of A¹ and B¹ is selected from the group consisting of hydrogen, halide, a substituted or unsubstituted C₁-C₂₀ alkyl group, a substituted or unsubstituted C₃-C₃₀ aryl group and a substituted or unsubstituted C₃-C₄₀ alkylaryl group, and the other of A¹ and B¹ has the formula:



wherein p is 0 or 1, X² is selected from the group consisting of -O-, -S-, -C(O)-, -S(O)₂- and -N(R)-, R is selected from the group comprising hydrogen, a substituted or unsubstituted C₁-C₂₀ alkyl group, a substituted or unsubstituted C₃-C₃₀ aryl group and a substituted or unsubstituted C₃-C₄₀ alkylaryl group, R⁶ and R⁷ are the same or different and are selected from the group consisting of hydrogen, a substituted or unsubstituted C₁-C₂₀ alkyl group, a substituted or unsubstituted C₃-C₃₀ aryl group and a substituted or unsubstituted C₃-C₄₀ alkylaryl group, and m is 0, 1 or 2. In this embodiment, B¹ preferably is selected from the group consisting of hydrogen, halide, a substituted or unsubstituted C₁-C₂₀ alkyl group, a substituted or unsubstituted C₃-C₃₀ aryl group and a substituted or unsubstituted C₃-C₄₀ alkylaryl group. Preferably, at least one, more preferably each, of R, R⁴, R⁵, R⁶ and R⁷ is hydrogen and preferably at least, more preferably both, of m and n are 1. It is further preferred that each of R¹, R² and R³ is hydrogen and that X¹ and X² are both -O-. Thus, in this embodiment, the most preferred form of linker moiety Z is derived from hydroquinone-O,O'-diacetic acid.

In yet another preferred embodiment, linker moiety Z has the following formula:



5

wherein R^4 , R^5 , R^6 and R^7 are the same or different and are selected from the group consisting of hydrogen, a substituted or unsubstituted C_1 - C_{20} alkyl group, a substituted or unsubstituted C_3 - C_{30} aryl group and a substituted or unsubstituted C_3 - C_{40} alkylaryl group, Y is selected from the group consisting of O, S, SO_2 and $\text{O}-((\text{CH}_2)_r-\text{O})_q$, l is an integer less than or equal to 60, q is an integer in the range of 1-1000, n and m are the same or different and are 1 or 2, with the proviso that, when Y is O, at least one of n and m is 2. Preferably, l is an integer in the range of 1-10, and q is an integer in the range of 1-1000. In this embodiment, the most preferred form of linker moiety Z is derived from thiodiglycolic acid (i.e. $\text{R}^4=\text{R}^5=\text{R}^6=\text{R}^7=\text{H}$, $n=m=1$ and $\text{Y}=\text{S}$).

The SUPPORT in the above formula is a conventional solid support. The nature of the solid support is not particularly restricted and is within the purview of a person skilled in the art. Thus, the solid support may be an inorganic substance. Non-limiting examples of suitable inorganic substances may be selected from the group consisting of silica, porous glass, aluminosilicates, borosilicates, metal oxides (e.g. aluminum oxide, iron oxide, nickel oxide) and clay containing one or more of these. Alternatively, the solid support may be an organic substance such as a cross-linked polymer. Non-limiting examples of a suitable cross-linked polymer may be selected from the group consisting of polyamide, polyether, polystyrene and mixtures thereof. The preferred solid support for use herein is conventional and may be selected from controlled pore glass bead or polystyrene beads. Further, the support may be either in particle form (e.g., beads), three-dimensional slabs (e.g., polymeric inserts and foams) or in a flat two-dimensional-like format (e.g., plastic sheets, glass chips, silicon wafers, etc.). The material used for the support may also be soluble in certain solvents (e.g., liquid-phase supports), but can be precipitated or crystallized from other solvents.

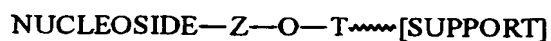
The reusable linker of formula:



5

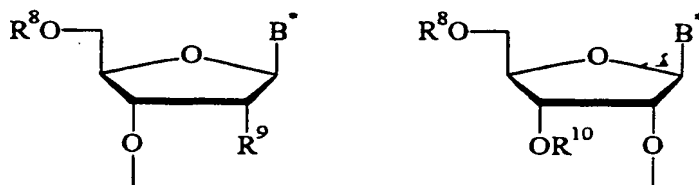
(again, Z is a linker moiety and T is an organic radical), may then be reacted with a conventional nucleoside-linker compound to produce another linker arm according to the present invention. This other linker arm has the following formula:

10

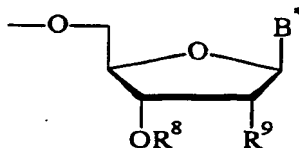


15 wherein Z is a linker moiety and T is an organic radical. The discussion herein above with respect to Z and T applies equally here. Preferably, in the above formula, NUCLEOSIDE is a moiety selected from one of the following formulae:

20



25



30

wherein R^8 and R^9 are the same or different and are hydrogen or a protecting group, R^9 is hydrogen (for deoxyribonucleosides or DNA) or $-OR^{11}$ (for ribonucleosides or RNA) wherein R^{11} is hydrogen or a protecting group, and B^* a nucleic acid base. Thus, in the case of RNA, there are two hydroxyl groups which may be protected. Also, the linker can be attached to either the 5', 3'- or (if ribose) 2'- hydroxyl positions. Indeed, for RNA sequences, it makes little difference whether the ester linker formed between the nucleoside and the linker compound is at the 2'- or 3'- hydroxyl position of the nucleoside. Thus, those of skill in the art will recognize that the nucleoside may be protected or blocked at the various of its hydroxyl moieties.

- 10 Non-limiting examples of useful protecting groups may be selected from the group consisting of acetyl, chloroacetyl, methoxyacetyl, t-butyl phenoxyacetyl, phenoxyacetyl, trityl, methoxytrityl, dimethoxytrityl (DMT), dialkylphosphite, pivalyl-isobutyloxycarbonyl, t-butyl dimethylsilyl, phenoxyacetal, 9-phenylxanthen-9-yl (pixyl), tetrahydropyranyl, methoxytetrahydropyranyl, methoxymethyl, benzyloxymethyl, methoxyethoxymethyl, methylthiomethyl, dialkylphosphate, levulinyl, dimethylphenylsilyl, trimethylsilyl, isopropyl dimethylsilyl, diisopropylmethylsilyl, diethylisopropylsilyl, triisopropylsilyl, acetyl, benzoyl, pivaloyl, trifluoroacetyl, allyl, benzyl, o-nitrobenzyl, o-hydroxystyryldimethylsilyl, 2-oxo-1,2-diphenylethyl, allyloxycarbonyl, monomethoxymethyl, nitroveratryloxycarbonyl, dimethoxybenzoin, dimethoxybenzoin carbonate, methyl nitropiperonyl carbonate, fluorenylmethoxycarbonyl, 2-phenylsulfonylethoxycarbonyl, fluorophenyl-methoxypiperidinyl and the like.
- 15
- 20

- As is known in the art, the main prerequisite for the protecting group used on the 5'-hydroxyl position is its ability to be selectively removed without causing cleavage of the linker arm. Thus, the preferred protecting group for desired 5'-hydroxyl position(s) is the acid labile dimethoxytrityl group. The main prerequisite for protecting groups on other hydroxyl positions, is stability to the conditions used for removal of the above protecting group. These latter protecting groups may be removed by the same conditions used to cleave the linker (discussed below) or separate conditions. The preferred protecting groups for these positions are trialkylsilyl (i.e. t-butyl dimethylsilyl) or acetyl.
- 25
- 30 Additional information may be obtained from the following references:

1. T. W. Greene and P. G. M. Nuts, "Protecting Groups in Organic Synthesis", Second Edition (1991), John Wiley and Sons, Inc., NY;
2. M. Schelhaas and H. Waldman, "Protecting Group Strategies in Organic Synthesis", *Angew. Chemie Int. Ed. Engl.* **35**, 2056-2083 (1996);
3. M. J. Gait, ed., "Oligonucleotide Synthesis A Practical Approach", IRL Press, Oxford (1984);
4. S. A. Narang, ed., "Synthesis and Applications of DNA and RNA", Academic Press, Inc., Orlando (1987); and
5. S. Agrawal, ed., "Methods in Molecular Biology, Vol. 20: Protocols for Oligonucleotides and Analogs", Humana Press, Totowa, NJ (1993);

the contents of each of which are hereby incorporated by reference, for a discussion of other possible hydroxyl protecting groups.

The manner by which the desired nucleoside may be protected is conventional and within the purview of a person skilled in the art. See, for example United States patent 3,400,190 (Melby), United States patent 4,458,066 (Caruthers et al.), the contents of each of which are hereby incorporated by reference.

A preferred method for production of deoxyribonucleosides in the context of the present invention is to use a nucleoside with a 5'-dimethoxytrityl protecting group and an appropriate exocyclic amino protecting group, e.g., N⁶-benzoyl-5'-dimethoxytrityl-2'-deoxyadenosine, N⁶-benzoyl-5'-dimethoxytrityl-2'-deoxycytidine, 5'-dimethoxytrityl-N²-isobutyryl-2'-deoxyguanosine, or 5'-dimethoxytritylthymidine.

A preferred method for production of ribonucleosides in the context of the present invention is to use a 5'-dimethoxytrityl protected nucleoside, with appropriate exocyclic amino protection, and no protecting groups on either of the 2'- or 3'- hydroxyl positions. The linker can then react with either one of the two adjacent hydroxyl groups (it doesn't matter which) to give a mixture of 2'- and 3'- linkages. The unreacted hydroxyl groups

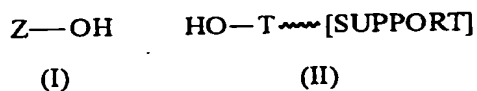
may then be acetylated by treatment of the immobilized nucleoside with acetic anhydride. Alternatively, ribonucleosides which have a 5'-dimethoxytrityl group, appropriate exocyclic amino group protection, and either a 3'-hydroxyl protecting group or a mixture of 2'- and 3'- protecting groups can be used. The 3'-protected compounds are generally unwanted isomers which are simultaneously produced when the 2'-hydroxyl position is protected and having little other use.

The reusable linker arm having the formula:



may be produced by a process comprising the step of reacting together the compound of Formulae I and II:

15

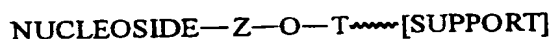


20

wherein Z and T are as defined above.

The reusable linker arm having the formula:

25



comprises the step of reacting together the compounds of Formulae I, II and III:



5

(I)

(II)



(III)

10

wherein Z and T are as defined above.

The compounds of Formulae I and II or of Formulae I, II and III (depending on which version of the present linker arm is being produced) are preferably reacted in the presence of an activating agent. As used throughout this specification, the term "activating group" is intended to have a broad meaning and is intended to encompass electrophilic reagents capable of activating a carboxyl moiety (e.g., on the linking compound of Formula II) by attachment of a leaving group to the acyl carbon of the carboxyl moiety - see, for example, M. Bodanszky, "Principles of Peptide Synthesis", Second Edition, Springer-Verlag, Berlin (1993), the contents of which are hereby incorporated by reference. Thus, the activating agent should be capable of initiating at least one of the following: (a) formation of a reactive acylating agent (this is an example of a derivate) from the carboxyl moiety in a separate step or steps, followed by immediate treatment with the amino component (in this case, for example, an amino-terminated support) to form an amide linkage or a hydroxy component (in this case a hydroxy-terminated support or a hydroxyl group on the desired nucleoside) to form an ester linkage; (b) formation of an isolable acylating agent, separately, optionally with purification prior to treatment with the amino or hydroxy component as discussed in (a); and (c) formation of an acylating intermediate in the presence of the amino/hydroxy component, by the addition of an activating agent to a mixture of the two components.

Thus, each of (a) and (c) are applicable to the formation of both carboxylic esters and amides and all three routes can be used to attach nucleosides to supports.

For example, the Letsinger method, which first reacts oxalyl chloride with triazole, and then adds a nucleoside to the resulting oxalyl triazolide is an example of route (a). Conversion of the carboxylic acid group into an "active" ester using either p-nitrophenol, or di-, tri-, tetra-, or penta-chlorinated or fluorinated phenols, or N-hydroxysuccinimide are common examples of route (b). Route (c) has been the most commonly used method in recent years and both the carbodiimide reagents (dicyclohexylcarbodiimide, 1-(3-dimethylaminopropyl)-ethylcarbodiimide, and diisopropylcarbodiimide) and uronium reagents (O-(7-azabenzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HATU), 2-(1H-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate, (HBTU)) may be used in this approach.

In a preferred embodiment, in addition to an activating reagent, the reaction of the compounds of Formulae I, II and III is conducted in the presence of a nucleophilic catalyst or additive (typically 4-dimethylamino pyridine (DMAP), 1-hydroxybenzotriazole (HOBt), or 1-hydroxy-7-azabenzotriazole (HOAt)) to speed up the reaction and a tertiary amine base (typically triethylamine, pyridine, or diisopropylethylamine) to ionize the carboxylic acid group.

Thus, those of skill in the art will recognize that the precise nature of the activation agent is not particularly restricted provided, of course, that the activated carboxylic acid group is capable of initiating formation of the ester or amide linkage, as appropriate, and the activating reagent does not have any deleterious effect on the desired nucleoside.

Thus, activation of the carboxylic acid by conversion into an acid chloride; an active ester (i.e., nitrophenyl, nitrophenylthio, trichlorophenyl, trifluorophenyl, pentachlorophenyl, pentafluorophenyl, or 3-hydroxy-2,3-dihydro-4-oxo-benzotriazine esters); an active hydroxylamine ester (i.e., N-hydroxyphthalimide or N-hydroxysuccinimide); acid anhydride; or mixed anhydride will produce derivatives which will form the desired linkage, and thus, these strategies are encompassed herein.

Non-limiting examples of activating agents may be selected from the group consisting of arylsulfonyl chlorides (e.g., benzenesulfonyl chloride (BS-Cl), mesitylenesulfonyl chloride (MS-Cl), triisopropylsulfonylchloride (TPS-Cl)); active arylsulfonyl esters (i.e., imidazole, triazole, nitrotriazole, or tetrazole esters of BS-Cl, MS-Cl or TPS-Cl); 2-ethoxy-1-(ethoxycarbonyl)-1,2-dihydroquinoline (EEDQ); acyl carbonates; 1,1'-(carbonyldioxy)dibenzotriazoles; chlorotrimethylsilane; carbodiimides (i.e., dicyclohexylcarbodiimide (DCC), 1-(3-dimethylaminopropyl)-ethylcarbodiimide (DEC), diisopropylcarbodiimide (DIC)) either alone or in combination with auxillary nucleophiles (i.e., 1-hydroxybenzotriazole (HOBt), 1-hydroxy-7-azabenzotriazole (HOAt), N-hydroxysuccinimide (HOSu), or 3-hydroxy-3,4-dihydro-1,2,3-benzotriazin-4-one (HOObt)) and/or catalysts (i.e., 4-dimethylaminopyridine (DMAP) or N-methylimidazole (NMI)); or uronium salts (i.e., tetramethyluronium chloride (TMU-Cl), 2-(1H-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HBTU), 2-(1H-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate (TBTU), 2-succinimido-1,1,3,3-tetramethyluronium tetrafluoroborate (TSTU), 2-(3,4-dihydro-4-oxo-1,2,3-benzotriazin-3-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate (TDBTU), 2-(2-oxo-1(2H)-pyridyl)-1,1,3,3-tetramethyluronium tetrafluoroborate (TPTU), 2-(5-norbornene-2,3-dicarboximido)-1,1,3,3-tetramethyluronium tetrafluoroborate (TNUT), O-(7-azabenzotriazol-1-yl)-1,3-dimethyl-1,3-dimethylenuronium hexa-fluorophosphate (HAMDU), O-(7-azabenzotriazol-1-yl)-1,3-dimethyl-1,3-trimethylenuronium hexafluorophosphate (HAMTU), O-(7-azabenzotriazol-1-yl)-1,1,3,3-bis(pentamethylene)uronium hexafluorophosphate (HAPipU), O-(7-azabenzotriazol-1-yl)-1,1,3,3-bis(tetramethylene)uronium hexafluorophosphate (HAPyU), O-(7-azabenzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HATU)) either alone or in combination with auxillary nucleophiles (i.e., 1-hydroxybenzotriazole (HOBt), 1-hydroxy-7-azabenzotriazole (HOAt), N-hydroxysuccinimide (HOSu), or 3-hydroxy-3,4-dihydro-1,2,3-benzotriazin-4-one (HOObt)) and/or catalysts (e.g. 4-dimethylaminopyridine (DMAP) or N-methylimidazole (NMI)) or phosphonium salts (e.g. benzotriazol-1-yl-oxytris(dimethylamino)phosphonium hexafluorophosphate (BOP), benzotriazole-1-yl-oxy-trispyrrolidinophosphonium hexafluorophosphate (PyBOP), 2-

(benzotriazol-1-yl)-1,3-dimethylimidazolidinium hexafluorophosphate (BOI), bromo tris(pyrrolidino)phosphonium hexafluorophosphate (PyBroP), 7-azabenzotriazol-1-yloxytris-(dimethylamino)phosphonium hexafluorophosphate (AOP), and 7-azabenzotriazol-1-yloxytris(pyrrolidino)phosphonium hexafluorophosphate (PyAOP))
5 either alone or in combination with auxillary nucleophiles and/or catalysts (discussed above) will also produce the desired linkage.

Other examples of suitable activating reagents may be found in any of the following references:

- 10 M. Bodanszky, "Principles of Peptide Synthesis", Second Edition, Springer-Verlag, Berlin (1993);
J. Jones, "Amino Acid and Peptide Synthesis", Oxford University Press, Oxford (1992);
G. Grant, "Synthetic Peptides: A Users Guide", W. H. Freeman & Co.,
15 NY (1992);
E. Haslam, Tetrahedron, 36, pg. 2409, (1980); and
M. A. Ogliaruso and J. F. Wolfe, "Synthesis of Carboxylic Acids, Esters and Their Derivatives", John Wiley & Sons, Chicester (1991);

20 the contents of each of which are hereby incorporated by reference.

In producing the present linker arm, the order of reaction is not particularly restricted. Thus, in one embodiment (this is the preferred embodiment), the compounds of Formulae I and III are initially reacted to form a conjugate which is reacted with the compound of Formula II. In another embodiment, the compounds of Formulae I and II
25 are initially reacted to form a conjugate which is reacted with the compound of Formula III.

The addition of compounds of Formulae I and III to Formula II, usually will not result in the quantitative conversion of each immobilized hydroxyl group into a derivatized ligand. Therefore, it is preferred that unreacted hydroxyl groups on the surface
30 of the support be protected (capped) by reaction with a capping reagent. This will mitigate

the free hydroxyl group participating in subsequent oligonucleotide chain extension reactions, resulting in defect sequences lacking the terminal nucleoside. Preferably, the capping reagent should be reversible so that the capping agent can be removed to regenerate the hydroxyl sites prior to the next round of support derivatization. Capping of the unreacted sites is conventional and can be performed by reaction with an activated carboxylic acid or anhydride to form an ester, or by addition of a protecting group, as described hereinabove. Thus, for example, t-butylphenoxyacetic anhydride, methoxyacetic anhydride or preferably chloroacetic anhydride, combined with 2,6-lutidine and N-methylimidazole in THF solution are useful examples of capping reagents.

With reference to Figure 2 there is illustrated a preferred pathway illustrating the use of the present linker arm in a recycled/regenerated manner. In Figure 2, DMT refers to dimethoxytrityl and B refers to a nucleobase as described hereinabove. As will be apparent to those of skill in the art, the support is recycled after oligonucleotide cleavage and support regeneration to a point in the reaction scheme where it may again be coupled with the HQPD-nucleoside conjugate for further oligonucleotide synthesis.

With further reference to "Oligo Synthesis" (Step #3) in Figure 2, once the present linker arm has been produced, it may be used in the conventional manner to synthesize an oligonucleotide - see, for example, United States patent 5,112,962 (Letsinger), incorporated by reference hereinabove. Once the oligonucleotide has been synthesized, it may be cleaved from the solid support to yield the free oligonucleotide and the support may then be regenerated - see Step #4 of Figure 2.

The cleavage step comprises hydrolysis at the point of attachment of the initial nucleoside to the linking compound. The regeneration of the support involves the removal of two moieties: (i) the removal of the structure represented by Formula I (above) from Formula II (above), which occurs simultaneously with the release of the oligonucleotide product, and (ii) the removal of the moiety used to protect (cap) unreacted hydroxyl sites of Formula II (above) on the support. Removal of these two moieties can occur simultaneously or separately to regenerate the support. Simultaneous removal of both moieties using only a single reagent is simpler but care should be taken to use reagents which will not deleteriously affect the oligonucleotide product. A two-step regeneration

involving the removal of the oligonucleotide using one reagent (usually ammonium hydroxide) and then treatment of the support with a second reagent (which may be faster but otherwise damaging to the oligonucleotide product thereby necessitating use of a two-step regeneration) allows flexibility in the choice of capping and regeneration reagents.

- 5 The reagent used to effect cleavage is not particularly restricted and is within the purview of a person skilled in the art. Preferably, the reagent is a base mild enough not to damage the oligonucleotide product but sufficiently strong to effect rapid cleavage. Non-limiting examples of suitable reagents for this purpose may be selected from the group consisting of ammonium hydroxide, ammonium hydroxide/methanol, 10 ammonia/methanol, ammonium hydroxide/methylamine, potassium carbonate/methanol, t-butylamine, ethylenediamine, methylamine, dimethylamine, trimethylamine/water and the like. Cleavage may also be performed under neutral conditions using fluoride ion (i.e. 1M tetrabutylammonium fluoride/THF or triethylamine trihydrofluoride). The reagent used to remove the capping reagent from unreacted sites may consist of the above reagents 15 or other stronger bases such as sodium or potassium hydroxide. In our preferred embodiment, ammonium hydroxide can be used to cleave the oligonucleotide product from the support, remove the HQPD linker arm, and cleave chloroacetyl protected hydroxyl groups in a single regeneration step. The preferred temperature for the cleavage and regeneration is room temperature, but higher or lower temperatures can be employed, 20 subject to the limitations of the apparatus used.

With reference to Figure 3, there are illustrated specific preferred examples of hydroxyl reusable linker arms falling within the scope of the present invention.

- Embodiments of the invention will be illustrated in the following Examples which should not be construed as limiting the scope of the invention. In the Examples, the 25 following materials were used:

1. Long chain alkylamine (LCAA) or glycerol (Gly) derivatized controlled pore glass (CPG) beads (120/200 mesh) were obtained from CPG Inc (Lincoln Park, NJ);
2. Toyopearl AF-amino-650M and HW65F supports were obtained from TosoHaas (Montgomeryville, PA);

3. Other supports were obtained from the manufacturers listed in Tables 1 and 2;

4. HQPD, Hydroquinone-O,O'-diacetic acid, commercially available from Lancaster Synthesis Ltd. (Lancashire, England);

5. Ammonium hydroxide solutions (28-30%) and solvents were obtained from VWR Canlab (Edmonton, Alberta, Canada);

6. Capping solutions were formulated as either Cap A (acetic anhydride/2, 6-lutidine/THF in a volume ratio of 1:1:8) and Cap B (N-methylimidazole and THF in a volume ratio of 16:84) or Cap A (chloroacetic anhydride and THF, 17% by weight) and Cap B (2, 6-lutidine and N-methylimidazole in THF in a volume ratio of 12:16:72);

7. Anhydrous pyridine and acetonitrile, distilled from CaH_2 ;

8. DIEA, diisopropylethylamine, reagent grade;

9. MeCN, acetonitrile, low water DNA synthesis grade;

10. DMAP, 4-dimethylaminopyridine, reagent grade;

11. DEC, 1-(3-dimethylaminopropyl)-ethylcarbodiimide, reagent grade;

12. Sulfurizing reagent, Beaucage thiolating reagent, from Pharmacia Biotech, was used as a 0.05M solution in acetonitrile; and

13. HBTU, 2-(1H-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate, reagent grade;

In the following Examples the amount of nucleoside (loading) on the insoluble supports was determined by spectrophotometric trityl analysis. In this procedure, a sample of support (4-5 mg) was accurately weighed directly into a 10 mL volumetric flask. A solution of dichloroacetic acid in 1,2-dichloroethane in a volume ration of 5:95 was then added to fill the flask. The contents were then thoroughly mixed and the absorbance of the orange coloured solution was measured at 503 nm using a Philips UV/Vis spectrophotometer. The nucleoside loading (in $\mu\text{mol/g}$ of CPG) was then calculated as:

$$\text{Loading} = (A_{503} \times \text{Vol} \times 1000) / (\text{Wt} \times 76)$$

wherein A_{503} = absorbance at 503 nm, Vol = solution volume in mL and Wt = amount of CPG tested in mg. The accuracy of the trityl determination was approximately $\pm 2-3\%$.

Example 1 - SYNTHESIS OF NUCLEOSIDE-3'-O-HODA HEMIESTERS

5 5'-Dimethoxytrityl-N-protected deoxyribonucleoside (10 mmol), hydroquinone-
O,O'-diacetic acid (15 mmol, 3.39 g), 4-dimethylaminopyridine (1 mmol, 122 mg), and
1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (15 mmol, 2.88 g) were
combined in a 100 mL round bottom flask equipped with a magnetic stir bar.
Triethylamine (0.8 mL) and anhydrous pyridine (50 mL) were added to the flask and the
10 contents were stirred at room temperature overnight.

The reaction was checked by TLC (5% methanol/chloroform). If more than a
trace of starting nucleoside was visible, more 1-(3-dimethylaminopropyl)-3-
ethylcarbodiimide hydrochloride (2-5 mmol) was added to the reaction and stirring was
continued for another day. When TLC showed complete disappearance of the starting
15 nucleoside, the solution was concentrated by evaporation until a thick oil was formed.
The oil was redissolved in chloroform (~ 200 mL) and transfer to a separatory funnel.
The chloroform solution was washed with aqueous sodium bicarbonate (~ 100 mL x 2)
and then water (~ 100 mL x 3). The funnel was slowly inverted to mix the two phases.
The chloroform phase was collected and the aqueous phase was discarded. If an
20 inseparable emulsion was formed, then either centrifugation (for small volumes) or (for
large volumes) precipitation by addition of hexanes followed by filtration and redissolving
the sticky precipitate back into chloroform can be performed.

The chloroform solution was added to anhydrous magnesium sulfate and mixed
to remove residual moisture from the solution. The magnesium sulfate was filtered off,
25 the filtrate was washed with a small amount of chloroform and then the chloroform
solution was evaporated to dryness. A light brown foam, containing a mixture of diester
and nucleoside hemiester sodium salt, was formed and solidified.

The hemiester sodium salt was converted into a more soluble pyridinium salt by
dissolving the foam in pyridine (~ 50-100 mL) and then adding AG 50W-X4 H⁺ cation
30 exchange resin (2 eq.). The mixture was stirred for approximately 5 minutes and then the

ion exchange resin was filtered off. The pyridine solution was evaporated to dryness. A light brown foam formed and solidified. The solid was dried under vacuum overnight to remove excess pyridine.

5 Example 2 - PREPARATION OF 12-DIMETHOXYTRITYL-HYDROXYDO-
DECANOIC ACID DERIVATIZED SUPPORTS

This example describes the synthesis of a C₁₂ linker arm within the scope of the present invention and how it can be used to convert commercially available amino-derivatized supports into reusable hydroxyl-derivatized supports.

- 10 12-Hydroxydodecanoic acid (9.25 mmol) was coevaporated to dryness with pyridine (3x). Then pyridine (~ 40 mL) and dimethoxytrityl chloride (10.2 mmol) were added. After stirring overnight, the solution was concentrated (to 10 mL), diluted with CHCl₃ (50 mL), washed with aq. NH₄HCO₃ (3x) and water (2x). The crude material was then purified on a silica gel column by elution with a 1% TEA/CHCl₃ - 4%
15 MeOH/1%TEA/CHCl₃ gradient. The product yield was 6.7 mmol (72%) of 12-dimethoxytrityl-hydroxydodecanoic acid as a brown oil.

- An amino functionalized support (0.5 g), 12-dimethoxytritylhydroxy-dodecanoic acid (0.2 mmol), 4-dimethylaminopyridine (0.1 mmol), 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (0.6 mmol), triethylamine (0.1 mL), and pyridine (7
20 mL) were shaken at room temperature (16 h). The support was filtered off, washed, and dried. Linker loading was determined by trityl analysis and the results are provided in Table 1. Unreacted amino and hydroxyl groups on the derivatized support (if present) were then acetylated by treating the support with equal volumes 1 M acetic anhydride/2,6-lutidine/THF (Cap A) and 2M N-methylimidazole/THF (Cap B) reagents
25 for 3 hours. The support was then filtered off, washed, and dried.

Table 1- Loading Results Using 12-Dimethoxy-
hydroxydodecanoic Acid Linker Arm

Experiment	Support	Linker arm loading ($\mu\text{mol/g}$)
1	Pharmacia HL-30 amino primer support	216
2	Long chain alkylamine CPG	87
3	Amino Tentagel, Millipore	107
4	Toyopearl AF-amino-650M	217
5	Aminoethyl polystyrene, Hamilton	73
6	Aminomethyl polystyrene, Applied Biosystems	28

**Example 3 - DERIVATIZATION OF TOYOPEARL HW-65F SUPPORT WITH
1,4-BUTANEDIOL DIGLYCIDYL ETHER**

This Example describes how hydroxyl surface groups on commercially available Toyopearl HW65 supports are extended with a butane diglycidyl linker to create a reusable support.

Toyopearl HW-65F vinyl alcohol/methacrylic acid copolymer was obtained as a slurry in 500 ml 20% ethanol/water. This slurry was evaporated to dryness to yield of 90 g of dry support. The hydroxyl content of the dry support was determined, in triplicate, by derivatization with dimethoxytrityl chloride/tetrabutylammonium perchlorate and trityl analysis, to be 1,095 $\mu\text{mol/g}$.

The dry HW-65F support (25 g), 1.0 M aqueous NaOH solution containing 1 mg/mL NaBH_4 (100 mL) and 1,4-butanediol diglycidyl ether (75 mL) were shaken at room temperature (3.5 h). The support was filtered off and washed with water, acetonitrile, and then chloroform. After drying, DMT derivatization and analysis (M.P. Reddy and P.J. Voelker, 1988, Int. J. Peptide Protein Res. 31, 345-348, the contents of which are hereby incorporated by reference) of a sample indicated 902 $\mu\text{mol/g}$ of remaining hydroxyl groups. Therefore, the epoxide loading was estimated to be 193 $\mu\text{mol/g}$.

The epoxide derivatized support (25 g), benzoic anhydride (51 g), 4-dimethylaminopyridine (6.6 g) and anhydrous pyridine (180 mL) were shaken at room temperature (overnight) to benzoylate unreacted hydroxyl groups. The support was filtered off, washed (methanol, then chloroform), and dried. DMT derivatization and analysis indicated that the residual hydroxyl group loading had decreased to only 5 $\mu\text{mol/g}$.

The benzoylated support (25 g), THF (140 mL), and 2.9 N aqueous HClO_4 (16.6 mL, 48 mmol) were shaken at room temperature (13 h). Trityl derivatization and analysis of an aliquot showed an hydroxyl loading of 98 $\mu\text{mol/g}$. Additional 2.9 N HClO_4 (34 mL) was added and shaking continued for another 3 h. The support was filtered off, washed, and dried and a final trityl derivatization and analysis indicated an hydroxyl loading of 103 $\mu\text{mol/g}$.

Example 4 - SYNTHESIS OF OLIGONUCLEOTIDE PHOSPHOROTHIOATES
AND SUPPORT RECYCLING USING CHLOROACETIC
ANHYDRIDE CAPPING.

This Example provides experiments which illustrate the suitability of a variety of different supports for repetitive oligonucleotide synthesis.

The following reagents were installed on a Perkin-Elmer/Applied Biosystems 394 4-column, 8-base position DNA synthesizer:

Ports #1-4: dA^{Bz} , dG^{Bz} , dC^{Bz} , and T phosphoramidites (0.2 M solutions).

Port #7: 0.15 M 5'-dimethoxytrityl- N^6 -benzoyl-2'-deoxyadenosine-3'-*O*-hydroquinone-*O,O'*-diacetyl hemiester pyridinium salt and 0.15 M diisopropylethylamine in anhydrous acetonitrile.

Port #8: 0.15 M HBTU and 0.15M DMAP in anhydrous acetonitrile.

Port #9: 0.45 M Tetrazole/acetonitrile.

Port #10: 2 M Ammonium hydroxide.

Port #11: 1 M Chloroacetic anhydride in THF (Cap A reagent).

5 Port #12: 1 M 2,6-Lutidine and 2 M N-methylimidazole in THF (Cap B reagent).

Port #14: 5% (v/v) Dichloroacetic acid/1,2-dichloroethane.

Port # 15: 0.05 M Beaucage reagent in acetonitrile.

10

Up to four synthesis columns, each containing one of the supports listed in Table 2, were installed on the synthesizer and, if necessary, manually detritylated to deblock the hydroxyl linker arm.

15 The synthesizer was then programmed to automatically execute the following steps:

- 1: A "Begin" procedure consisting of a column wash, nucleoside coupling to the support by simultaneous addition (4.0 sec) of nucleoside hemiester (port #7) and coupling reagent (port #8) and a 600 sec wait, column wash, capping of unreacted hydroxyl sites (Cap A + B reagents, 300 sec), column wash, and priming of ports #1, 2, 3, 4, and 9.
- 20
- 2: Synthesis of the 20-base phosphorothioate oligonucleotide sequence dGCCCAAGCTGGCATCCGTCA (Trityl-off).
- 25
- 3: A 15 minute ammonium hydroxide hydrolysis step to cleave the oligonucleotide from the support.

After completion of the ammonium hydroxide hydrolysis, the columns were removed from the synthesizer, manually treated with 0.05 M potassium carbonate/methanol solution (5 min), rinsed with methanol, dried by aspiration (5 min), re-installed on the synthesizer, and rinsed with anhydrous acetonitrile. The automated synthesis was then repeated (i.e., Steps 1, 2, and 3 above) using the same synthesis column a total of twelve times.

The amount of trityl color released after the first detritylation step was collected and quantitated to determine the amount of nucleoside added to the support - the results are reported in Table 3. The released oligonucleotide solution was deprotected (55°C, 16 h), evaporated to removed ammonia, and quantitated by UV at 260 nm - the results are reported in Table 4. The correct identity of the products, obtained from each of the results shown Table 4, was verified by electrophoresis and comparison to authentic material. Furthermore, no unusual impurities, attributable to the support recycling were present. These results confirmed that each of the nine supports used in this experiment could be reused and in several cases satisfactory results (comparable to new supports) were obtained, even after six or more uses.

Example 5 - SYNTHESIS OF OLIGONUCLEOTIDE PHOSPHOROTHIOATES
AND SUPPORT RECYCLING USING METHOXYACETIC
ANHYDRIDE CAPPING

This Example illustrates the use of methoxyacetic anhydride as the capping reagent instead of chloroacetic anhydride used in the previous Examples.

The automated DNA synthesizer was set-up with reagents, as described in Example 4, with the exception of the Cap A and B reagents, which were as follows:

Port #10: 0.5 M Methoxyacetic anhydride and 0.5 M 2,6-lutidine in acetonitrile (Cap A).

Port #12: 1 M N-Methylimidazole in acetonitrile (Cap B).

The automated nucleoside derivatization, oligonucleotide synthesis, and support recycling procedure was then performed using the supports listed in Table 5 and the procedure described in Example 4. However, because of the greater stability of the methoxyacetyl group, the manual column regeneration step with 0.05M potassium carbonate/methanol was increased from 5 min to 15 min.

The amount of trityl color released after the first detritylation step was collected and quantitated to determine the amount of nucleoside added to the support - the results are reported in Table 6. The released oligonucleotide solution was deprotected (55°C, 16 h), evaporated to remove ammonia, and quantitated by UV at 260 nm - the results are reported in Table 7. The composition of the products obtained in Table 7 was examined by gel electrophoresis and the expected products were obtained in each case. This indicated that methoxyacetic anhydride could also be used as a satisfactory capping reagent during the support recycling.

Table 2 - Supports Used For Phosphorothioate Synthesis and Support Recycling

Experiment	Support	Linker Arm	Amount used (mg)
1	Long chain alkylamine CPG	Hydroxyhexylsuccinylidiamide	16.2
2	Long chain alkylamine CPG	Hydroxydodecanoic acid	21.1
3	Glycerol CPG		13.3
4	Toyopearl AF-amino-650M	Hydroxydodecanoic acid	15.0
5	Aminoethyl polystyrene, Hamilton	Hydroxydodecanoic acid	21.5
6	Aminomethyl polystyrene, Applied Biosystems	Hydroxydodecanoic acid	34.1
7	Pharmacia hydroxyl primer support*	Butanediol diglycidyl	14.5
8	Toyopearl HW65F	Butanediol diglycidyl	15.4
9	Hydroxyethyl polymethacrylate/polystyrene, Hamilton		27.3

*proprietary material supplied by Pharmacia

Table 3 - Nucleoside loading obtained after repetitive synthesis on the same support

Experiment	Synthesis # and First Nucleoside Loading Level (µmol/g)											
	1	2	3	4	5	6	7	8	9	10	11	12
1	74	62	61	65	56	55	54	60	58	60	51	49
2	57	58	54	56	54	54	54	62	51	49	56	46
3	72	68	65	67	64	64	62	61	60	57	55	54
4	96	119	130	130	126	123	65	60	46	34	25	17
5	75	79	76	76	70	57	47	40	43	60	46	40
6	30	35	35	37	38	35	35	36	31	33	37	33
7	155	111	107	97	122	115	95	122	87	110	101	89
8	107	110	116	113	111	104	102	87	76	92	71	66
9	34	41	47	48	48	53	49	50	45	62	51	67

Table 4 - Amount of Crude Oligonucleotide Produced From Repetitive Syntheses on the Same Support

Experiment	Synthesis # and amount of crude product produced (A_{260} units)											
	1	2	3	4	5	6	7	8	9	10	11	12
1	7530	7590	7530	7650	6790	6850	5740	5930	4750	3640	2780	2350
2	7630	7680	6590	6970	6680	6680	6110	5170	3460	2420	2700	1850
3	8350	7740	8270	7970	8120	8120	7440	7370	6690	6170	5190	4660
4	12200	11500	12600	11700	11600	11800	9880	6710	5850	4750	3540	2550
5	8010	8340	7990	7960	7810	6720	5690	3900	3280	3780	2550	2050
6	3610	4300	4220	4540	4830	4560	4630	4620	4300	3730	3390	2750
7	9300	8190	7460	7620	8550	7390	5900	4900	2280	4180	2430	1750
8	11520	11100	10600	11400	11500	11400	9980	n.d.	n.d.	8390	5060	6270
9	2360	3260	4520	4630	5290	6420	6610	n.d.	n.d.	8570	6420	6830

Table 5 - Supports Used For Oligonucleotide Phosphorothioate Synthesis and Support Recycling

Experiment	Support	Linker Arm	Amount used (mg)
1	Glycerol CPG		14.5
2	Toyopearl HW65F	Butanediol diglycidyl	14.6

Table 6 - Nucleoside Loading Obtained After Repetitive Synthesis on the Same Support

Experiment	Synthesis # and first nucleoside loading ($\mu\text{mol/g}$)											
	1	2	3	4	5	6	7	8	9	10	11	12
1	83	82	71	75	61	69	63	64	58	56	48	42
2	93	105	115	115	105	114	117	120	110	113	96	67

Table 7 - Amount of Crude Oligonucleotide Produced From Repetitive Syntheses on the Same Support

Experiment	Synthesis # and amount of crude product produced (A_{260} units)											
	1	2	3	4	5	6	7	8	9	10	11	12
1	8100	7750	7570	7850	6910	7500	7440	7400	6870	6810	6210	5220
2	8480	9820	10200	10900	9490	10200	10300	10700	10000	9190	8450	6170

What is claimed is:

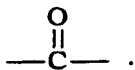
1. A reusable linker arm for solid support oligonucleotide synthesis, the linker arm comprising the following formula:



wherein Z is a linker moiety and T is an organic radical.

2. The reusable linker arm defined in claim 1, wherein T contains at least one carbon.
3. The reusable linker arm defined in claim 1, wherein T is a C_1-C_{300} organic moiety.
4. The reusable linker arm defined in claim 1, wherein T is a C_1-C_{200} organic moiety.
5. The reusable linker arm defined in claim 1, wherein T is a C_1-C_{100} organic moiety.
6. The reusable linker arm defined in claims 1-5, wherein T is a saturated organic moiety.
7. The reusable linker arm defined in claims 1-5, wherein T is an unsaturated organic moiety.
8. The reusable linker arm defined in claim 1, wherein T is a C_1-C_{300} organic moiety comprising at least one heteroatom selected from N and O.

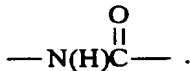
9. The reusable linker arm defined in claims 1-8, wherein the organic moiety comprises at least one moiety having the formula:



10. The reusable linker arm defined in claims 1-8, wherein the organic moiety comprises at least one moiety having the formula:



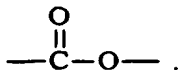
11. The reusable linker arm defined in claims 1-8, wherein the organic moiety comprises at least one moiety having the formula:



12. The reusable linker arm defined in claims 1-8, wherein the organic moiety comprises at least one moiety having the formula:



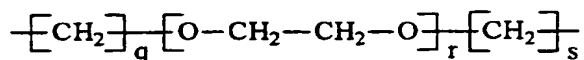
13. The reusable linker arm defined in claims 1-8, wherein organic moiety comprises at least one moiety having the formula:



14. The reusable linker arm defined in claims 1-13, wherein the organic moiety is unsubstituted.

15. The reusable linker arm defined in claim 14, wherein the organic moiety is substituted by at least one moiety selected from the group comprising a C₁-C₄₀ alkyl group, a C₅-C₄₀ aryl group, a C₁-C₄₀ alkoxy group, a C₁-C₄₀ ester group, a C₁-C₄₀ hydroxy group, a C₂-C₄₀ acrylate group and a C₃-C₄₀ alkylaryl group.

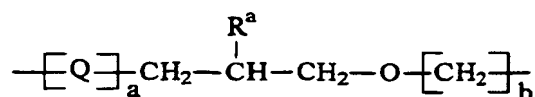
16. The reusable linker arm defined in claims 1-15, wherein T has the formula:



wherein q and s are the same or different and each is an integer having a value of 0-40 and r is an integer having a value of 1-200.

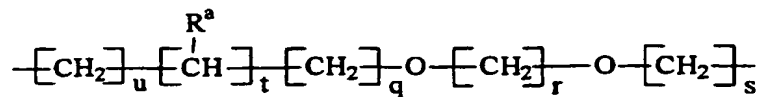
17. The reusable linker arm defined in claim 16, wherein q and s are the same or different and each is an integer having a value of 1-20 and r is an integer having a value of 1-150.

18. The reusable linker arm defined in claims 1-15, wherein T has the formula:



wherein a is 0 or 1, Q is an organic moiety, R^a is selected from -OH, -NH₂, -NR and -OR wherein R is a protecting group and b is an integer having a value of 0-40.

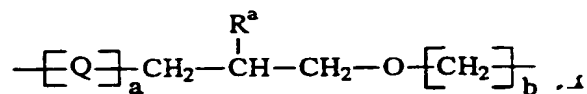
19. The reusable linker arm defined in claim 18, wherein a is 0 and R^a is -OH.
20. The reusable linker arm defined in claim 18, wherein a is 1 and R^a is -NR or -OR.
21. The reusable linker arm defined in claims 18-20, wherein the protecting group is selected from the group comprising acetyl, chloroacetyl, methoxyacetyl, t-butyl phenoxyacetyl, phenoxyacetyl, trityl, methoxytrityl, dimethoxytrityl (DMT), dialkylphosphite, pivalyl-isobutyloxycarbonyl, t-butyl dimethylsilyl, 9-phenylxanthene-9-yl (pixyl), tetrahydropyranyl, methoxytetrahydropyranyl, methoxymethyl, benzyloxymethyl, methoxyethoxymethyl, methylthiomethyl, dialkylphosphate, levulinyl, dimethylphenylsilyl, trimethylsilyl, isopropyl dimethylsilyl, diisopropyl methylsilyl, diethylisopropylsilyl, triisopropylsilyl, benzoyl, pivaloyl, trifluoroacetyl, allyl, benzyl, o-nitrobenzyl, o-hydroxystyryl dimethylsilyl, 2-oxo-1,2-diphenylethyl, allyloxycarbonyl, monomethoxymethyl, nitroveratryloxycarbonyl, dimethoxybenzoin, dimethoxybenzoin carbonate, methyl nitropiperonyl carbonate, fluorenylmethoxycarbonyl, 2-phenylsulfonyl-ethoxycarbonyl, fluorophenyl-methoxypiperidinyl and mixtures thereof.
22. The reusable linker arm defined in claim 18, wherein Q comprises a moiety having the formula:



wherein q, r, s, t and u are the same or different and each is an integer having a value of 0-40 and R^a is selected from the group comprising hydrogen, hydroxyl, a C₁-C₄₀ alkyl group, a C₅-C₄₀ aryl group, a C₁-C₄₀ alkoxy group, a C₁-C₄₀ ester group, a C₁-C₄₀ hydroxy group, a C₂-C₄₀ acrylate group, a C₃-C₄₀ alkylaryl group, -NH₂, -NHR and -OR, wherein R is a protecting group.

23. The reusable linker arm defined in claim 22, wherein s is 0, q, r and u are the same or different and each is an integer having a value of 1-10, t is an integer of 1-5 and R^a is hydroxyl.

24. The reusable linker arm defined in claims 1-15, wherein T has the formula:



wherein a is 0 or 1, Q is an organic moiety, R^a is selected from -OH, -NH₂, -NR and -OR wherein R is a protecting group and b is an integer having a value of 0-40.

25. The reusable linker arm defined in claim 24, wherein a is 0 and R^a is -OH.

26. The reusable linker arm defined in claim 24, wherein a is 1 and R^a is -NR or -OR.

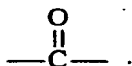
27. The reusable linker arm defined in claim 18, wherein Q is a C₁-C₁₀₀ organic moiety.

28. The reusable linker arm defined in claim 18, wherein Q is a saturated organic moiety.

29. The reusable linker arm defined in claim 18, wherein Q is an unsaturated organic moiety.

30. The reusable linker arm defined in claim 18, wherein T is a C₁-C₁₀₀ organic moiety comprising at least one heteroatom selected from N and O.

31. The reusable linker arm defined in claims 27-30, wherein the organic moiety comprises at least one moiety having the formula:



32. The reusable linker arm defined in claims 27-30, wherein the organic moiety comprises at least one moiety having the formula:



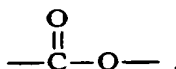
33. The reusable linker arm defined in claims 27-30, wherein the organic moiety comprises at least one moiety having the formula:



34. The reusable linker arm defined in claims 27-30, wherein the organic moiety comprises at least one moiety having the formula:



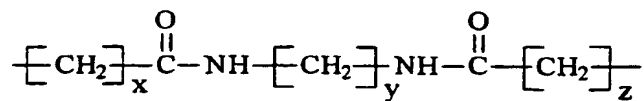
35. The reusable linker arm defined in claims 27-30, wherein organic moiety comprises at least one moiety having the formula:



36. The reusable linker arm defined in claim 27-35, wherein the organic moiety is unsubstituted.

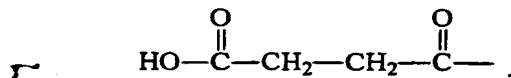
37. The reusable linker arm defined in claim 27-35, wherein the organic moiety is substituted by at least one moiety selected from the group comprising a C₁-C₄₀ alkyl group, a C₃-C₄₀ aryl group, a C₁-C₄₀ alkoxy group, a C₁-C₄₀ ester group, a C₁-C₄₀ hydroxy group, a C₂-C₄₀ acrylate group and a C₃-C₄₀ alkylaryl group.

38. The reusable linker arm defined in claim 18, wherein Q has the formula:

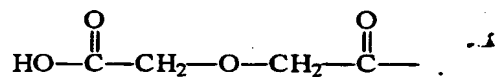


wherein each of x, y and z is an integer having a value of 1-40.

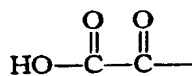
39. The reusable linker arm defined in claims 1-38, wherein Z has the following formula:



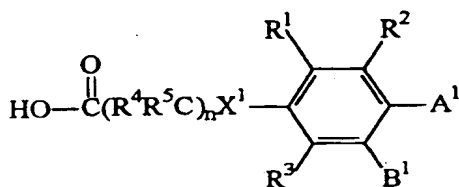
40. The reusable linker arm defined in claims 1-38, wherein Z has the following formula:



41. The reusable linker arm defined in claims 1-38, wherein Z has the following formula:

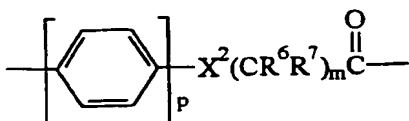


42. The reusable linker arm defined in claims 1-38, wherein Z has the following formula:



wherein: R^1 , R^2 and R^3 are the same or different and are selected from the group consisting of hydrogen, halide, a substituted or unsubstituted $\text{C}_1\text{-C}_{20}$ alkyl group, a substituted or unsubstituted $\text{C}_3\text{-C}_{30}$ aryl group and a substituted or unsubstituted $\text{C}_3\text{-C}_{40}$ alkylaryl group; R^4 and R^5 are the same or different and are selected from the group consisting of hydrogen, a substituted or unsubstituted $\text{C}_1\text{-C}_{20}$ alkyl group, a substituted or unsubstituted $\text{C}_3\text{-C}_{30}$ aryl group and a substituted or unsubstituted $\text{C}_3\text{-C}_{40}$ alkylaryl group; X^1 is selected from the group consisting of $-\text{O}-$, $-\text{S}-$, $-\text{C}(\text{O})-$, $-\text{S}(\text{O})_2-$ and $-\text{N}(\text{R})-$; R is selected from the group comprising hydrogen, a substituted or unsubstituted $\text{C}_1\text{-C}_{20}$ alkyl group, a substituted or unsubstituted $\text{C}_3\text{-C}_{30}$ aryl group and a substituted or unsubstituted $\text{C}_3\text{-C}_{40}$ alkylaryl group; n is 0, 1 or 2; and one of A^1 and B^1 is selected from the group consisting of hydrogen, halide, a substituted or unsubstituted $\text{C}_1\text{-C}_{20}$ alkyl

group, a substituted or unsubstituted C₃-C₃₀ aryl group and a substituted or unsubstituted C₃-C₄₀ alkylaryl group, and the other of A¹ and B¹ has the formula:



wherein p is 0 or 1, X² is selected from the group consisting of -O-, -S-, -C(O)-, -S(O)₂- and -N(R)-, R is selected from the group comprising hydrogen, a substituted or unsubstituted C₁-C₂₀ alkyl group, a substituted or unsubstituted C₃-C₃₀ aryl group and a substituted or unsubstituted C₃-C₄₀ alkylaryl group, R⁶ and R⁷ are the same or different and are selected from the group comprising hydrogen, a substituted or unsubstituted C₁-C₂₀ alkyl group, a substituted or unsubstituted C₃-C₃₀ aryl group and a substituted or unsubstituted C₃-C₄₀ alkylaryl group, and m is 0, 1 or 2.

43. The reusable linker arm defined in claim 42, wherein p is 0.

44. The reusable linker arm defined in claims 42-43, wherein B¹ is selected from the group consisting of hydrogen, halide, a substituted or unsubstituted C₁-C₂₀ alkyl group, a substituted or unsubstituted C₃-C₃₀ aryl group and a substituted or unsubstituted C₃-C₄₀ alkylaryl group.

45. The reusable linker arm defined in claims 42-44, wherein each of R⁴, R⁵, R⁶ and R⁷ is hydrogen.

46. The reusable linker arm defined in claims 42-45, wherein each of m and n are 1.

47. The reusable linker arm defined in claims 42-46, wherein each of R¹, R² and R³ is hydrogen.

48. The reusable linker arm defined in claims 42-47, wherein X^1 and X^2 are both -O-.

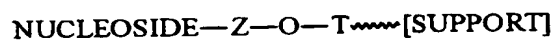
49. The reusable linker arm defined in claims 1-48, wherein SUPPORT is an inorganic substance.

50. The reusable linker arm defined in claim 49, wherein the inorganic substance is selected from the group consisting of silica, glass beads, porous glass, aluminosilicates, borosilicates, metal oxides, clays and mixtures thereof.

51. The reusable linker arm defined in claims 1-48, wherein SUPPORT is an organic substance.

52. The reusable linker arm defined in claim 51, wherein the organic substance is a cross-linked polymer.

53. A reusable linker arm for solid support oligonucleotide synthesis, the linker arm comprising the following formula:



wherein Z is a linker moiety and T is an organic radical.

54. The reusable linker arm defined in claim 53, wherein T contains at least one carbon.

55. The reusable linker arm defined in claim 53, wherein T is a C_1 - C_{300} organic moiety.

56. The reusable linker arm defined in claim 53, wherein T is a C₁-C₂₀₀ organic moiety.

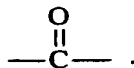
57. The reusable linker arm defined in claim 53, wherein T is a C₁-C₁₀₀ organic moiety.

58. The reusable linker arm defined in claims 53-57, wherein T is a saturated organic moiety.

59. The reusable linker arm defined in claims 53-57, wherein T is an unsaturated organic moiety.

60. The reusable linker arm defined in claims 53-57, wherein T is a C₁-C₂₀₀ organic moiety comprising at least one heteroatom selected from N and O.

61. The reusable linker arm defined in claims 53-60, wherein the organic moiety comprises at least one moiety having the formula:



62. The reusable linker arm defined in claims 53-60, wherein the organic moiety comprises at least one moiety having the formula:



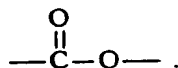
63. The reusable linker arm defined in claims 53-60, wherein the organic moiety comprises at least one moiety having the formula:



64. The reusable linker arm defined in claims 53-60, wherein the organic moiety comprises at least one moiety having the formula:



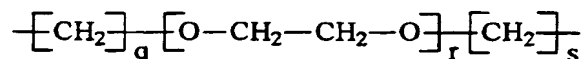
65. The reusable linker arm defined in claims 53-60, wherein organic moiety comprises at least one moiety having the formula:



66. The reusable linker arm defined in claims 53-65, wherein the organic moiety is unsubstituted.

67. The reusable linker arm defined in claims 53-65, wherein the organic moiety is substituted by at least one moiety selected from the group comprising a C₁-C₄₀ alkyl group, a C₃-C₄₀ aryl group, a C₁-C₄₀ alkoxy group, a C₁-C₄₀ ester group, a C₁-C₄₀ hydroxy group, a C₂-C₄₀ acrylate group and a C₃-C₄₀ alkylaryl group.

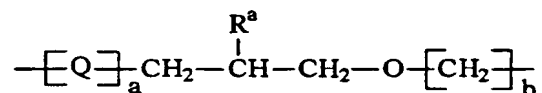
68. The reusable linker arm defined in claims 53-67, wherein T has the formula:



wherein q and s are the same or different and each is an integer having a value of 0-40 and r is an integer having a value of 1-200.

69. The reusable linker arm defined in claim 68, wherein q and s are the same or different and each is an integer having a value of 1-20 and r is an integer having a value of 1-150.

70. The reusable linker arm defined in claims 53-67, wherein T has the formula:



wherein a is 0 or 1, Q is an organic moiety, R^a is selected from -OH, -NH₂, -NR and -OR wherein R is a protecting group and b is an integer having a value of 0-40.

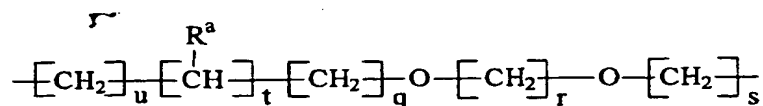
71. The reusable linker arm defined in claim 70, wherein a is 0 and R^a is -OH.

72. The reusable linker arm defined in claim 70, wherein a is 1 and R^a is -NR or -OR.

73. The reusable linker arm defined in claims 70-72, wherein the protecting group is selected from the group comprising acetyl, chloroacetyl, methoxyacetyl, t-butyl phenoxyacetyl, trityl, methoxytrityl, dimethoxytrityl (DMT), dialkylphosphite, pivalyl-

isobutyloxycarbonyl, *t*-butyldimethylsilyl, phenoxyacetal, 9-phenylxanthen-9-yl (pixyl), tetrahydropyranyl, methoxytetrahydropyranyl, methoxymethyl, benzyloxymethyl, methoxyethoxymethyl, methylthiomethyl, dialkylphosphate, levulinyl, dimethylphenylsilyl, trimethylsilyl, isopropyldimethylsilyl, diisopropylmethylsilyl, diethylisopropylsilyl, triisopropylsilyl, benzoyl, pivaloyl, trifluoroacetyl, allyl, benzyl, *o*-nitrobenzyl, *o*-hydroxystyryldimethylsilyl, 2-oxo-1,2-diphenylethyl, allyloxycarbonyl, monomethoxymethyl, nitroveratryloxycarbonyl, dimethoxybenzoin, dimethoxybenzoin carbonate, methylnitropiperonyl carbonate, fluorenylmethoxycarbonyl, 2-phenylsulfonyl-ethoxycarbonyl, fluorophenyl-methoxypiperidinyl and mixtures thereof.

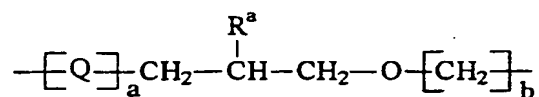
74. The reusable linker arm defined in claim 70, wherein Q comprises a moiety having the formula:



wherein q, r, s, t and u are the same or different and each is an integer having a value of 0-40 and R^a is selected from the group comprising hydrogen, hydroxyl, a C₁-C₄₀ alkyl group, a C₅-C₄₀ aryl group, a C₁-C₄₀ alkoxy group, a C₁-C₄₀ ester group, a C₁-C₄₀ hydroxy group, a C₂-C₄₀ acrylate group, a C₅-C₄₀ alkylaryl group, -NH₂, -NHR^a and -OR, wherein R is a protecting group.

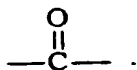
75. The reusable linker arm defined in claim 74, wherein s is 0, q, r and u are the same or different and each is an integer having a value of 1-10, t is an integer of 1-5 and R^a is hydroxyl.

76. The reusable linker arm defined in claim 70, wherein T has the formula:



wherein a is 0 or 1, Q is an organic moiety, R^a is selected from -OH, -NH₂, -NR and -OR wherein R is a protecting group and b is an integer having a value of 0-40.

77. The reusable linker arm defined in claim 76, wherein a is 0 and R^a is -OH.
78. The reusable linker arm defined in claim 76, wherein a is 1 and R^a is -NR or -OR.
79. The reusable linker arm defined in claims 53-78, wherein Q is a C₁-C₁₀₀ organic moiety.
80. The reusable linker arm defined in claims 53-78, wherein Q is a saturated organic moiety.
81. The reusable linker arm defined in claims 53-78, wherein Q is an unsaturated organic moiety.
82. The reusable linker arm defined in claims 53-78, wherein T is a C₁-C₁₀₀ organic moiety comprising at least one heteroatom selected from N and O.
83. The reusable linker arm defined in claims 76-82, wherein the organic moiety comprises at least one moiety having the formula:



84. The reusable linker arm defined in claims 76-82, wherein the organic moiety comprises at least one moiety having the formula:



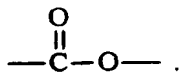
85. The reusable linker arm defined in claims 76-82, wherein the organic moiety comprises at least one moiety having the formula:



86. The reusable linker arm defined in claims 76-82, wherein the organic moiety comprises at least one moiety having the formula:



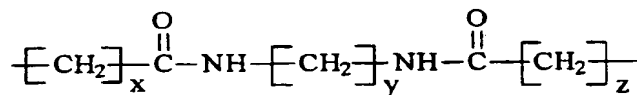
87. The reusable linker arm defined in claims 76-82, wherein organic moiety comprises at least one moiety having the formula:



88. The reusable linker arm defined in claims 76-87, wherein the organic moiety is unsubstituted.

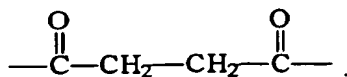
89. The reusable linker arm defined in claims 76-87, wherein the organic moiety is substituted by at least one moiety selected from the group comprising a C₁-C₄₀ alkyl group, a C₅-C₄₀ aryl group, a C₁-C₄₀ alkoxy group, a C₁-C₄₀ ester group, a C₁-C₄₀ hydroxy group, a C₂-C₄₀ acrylate group and a C₅-C₄₀ alkylaryl group.

90. The reusable linker arm defined in claim 53, wherein Q has the formula:

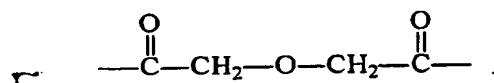


wherein each of x, y and z is an integer having a value of 1-40.

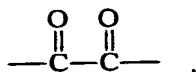
91. The reusable linker arm defined in claims 53-90, wherein Z has the following formula:



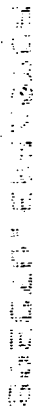
92. The reusable linker arm defined in claims 53-90, wherein Z has the following formula:



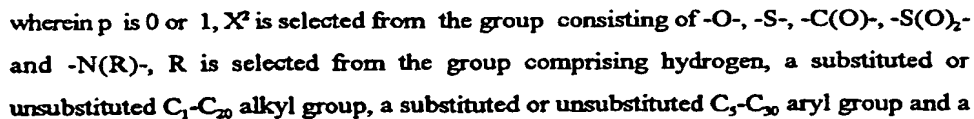
93. The reusable linker arm defined in claims 53-90, wherein Z has the following formula:



94. The reusable linker arm defined in claims 53-90, wherein Z has the following formula:



—



substituted or unsubstituted C₃-C₄₀ alkylaryl group, R⁶ and R⁷ are the same or different and are selected from the group comprising hydrogen, a substituted or unsubstituted C₁-C₂₀ alkyl group, a substituted or unsubstituted C₃-C₃₀ aryl group and a substituted or unsubstituted C₃-C₄₀ alkylaryl group, and m is 0, 1 or 2.

95. The reusable linker arm defined in claim 94, wherein p is 0.

96. The reusable linker arm defined in claims 94-95, wherein B¹ is selected from the group consisting of hydrogen, halide, a substituted or unsubstituted C₁-C₂₀ alkyl group, a substituted or unsubstituted C₃-C₃₀ aryl group and a substituted or unsubstituted C₃-C₄₀ alkylaryl group.

97. The reusable linker arm defined in claims 94-96, wherein each of R⁴, R⁵, R⁶ and R⁷ is hydrogen.

98. The reusable linker arm defined in claims 94-97, wherein each of m and n are 1.

99. The reusable linker arm defined in claims 94-98, wherein each of R¹, R² and R³ is hydrogen.

100. The reusable linker arm defined in claims 94-99, wherein X¹ and X² are both -O-.

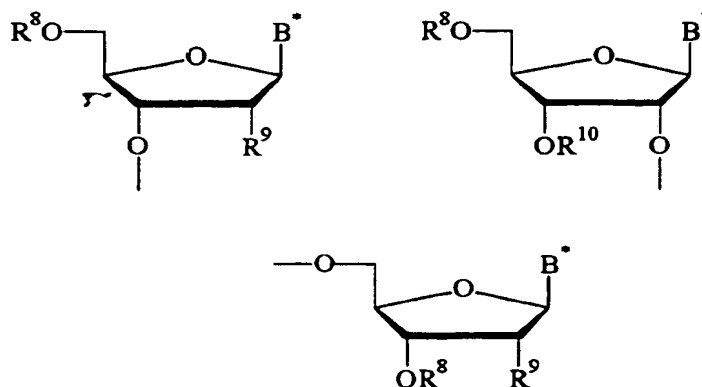
101. The reusable linker arm defined in claims 53-100, wherein SUPPORT is an inorganic substance.

102. The reusable linker arm defined in claim 101, wherein the inorganic substance is selected from the group consisting of silica, glass beads, porous glass, aluminosilicates, borosilicates, metal oxides, clays and mixtures thereof.

103. The reusable linker arm defined in claims 53-100, wherein SUPPORT is an organic substance.

104. The reusable linker arm defined in claim 103, wherein the organic substance is a cross-linked polymer.

105. The reusable linker arm defined in claims 53-104, wherein NUCLEOSIDE is a moiety selected from one of the following formulae:

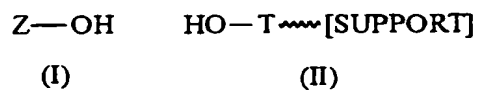


wherein R^8 and R^{10} are the same or different and are hydrogen or a protecting group, R^9 is hydrogen or $-OR^{11}$ wherein R^{11} is hydrogen or a protecting group, and B^+ is a nucleic acid base.

106. A process for production of a reusable linker arm for oligonucleotide synthesis having the following formula:



wherein Z is a linker moiety and T is an organic radical, the process comprising the step of reacting together the compound of Formulae I and II:

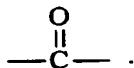


wherein Z and T are as defined above.

107. The process defined in claim 106, wherein T contains at least one carbon.
108. The process defined in claim 106, wherein T is a C_1 - C_{300} organic moiety.
109. The process defined in claim 106, wherein T is a C_1 - C_{200} organic moiety.
110. The process defined in claim 106, wherein T is a C_1 - C_{100} organic moiety.
111. The process defined in claims 106-110, wherein T is a saturated organic moiety.
112. The process defined in claims 106-110, wherein T is an unsaturated organic moiety.

113. The process defined in claims 106-112, wherein T is a C₁-C₃₀₀ organic moiety comprising at least one heteroatom selected from N and O.

114. The process defined in claims 106-113, wherein the organic moiety comprises at least one moiety having the formula:



115. The process defined in claims 106-113, wherein the organic moiety comprises at least one moiety having the formula:



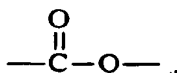
116. The process defined in claims 106-113, wherein the organic moiety comprises at least one moiety having the formula:



117. The process defined in claims 106-113, wherein the organic moiety comprises at least one moiety having the formula:



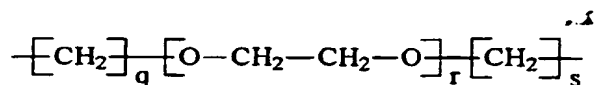
118. The process defined in claims 106-113, wherein organic moiety comprises at least one moiety having the formula:



119. The process defined in claims 106-118, wherein the organic moiety is unsubstituted.

120. The process defined in claims 106-118, wherein the organic moiety is substituted by at least one moiety selected from the group comprising a C₁-C₄₀ alkyl group, a C₃-C₄₀ aryl group, a C₁-C₄₀ alkoxy group, a C₁-C₄₀ ester group, a C₁-C₄₀ hydroxy group, a C₂-C₄₀ acrylate group and a C₃-C₄₀ alkylaryl group.

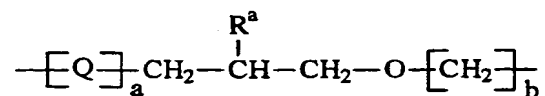
121. The process defined in claims 106-120, wherein T has the formula:



wherein q and s are the same or different and each is an integer having a value of 0-40 and r is an integer having a value of 1-200.

122. The process defined in claim 121, wherein q and s are the same or different and each is an integer having a value of 1-20 and r is an integer having a value of 1-150.

123. The process defined in claims 106-120, wherein T has the formula:



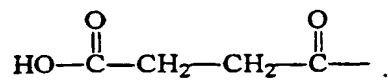
wherein a is 0 or 1, Q is an organic moiety, R^a is selected from -OH, -NH₂, -NR and -OR wherein R is a protecting group and b is an integer having a value of 0-40.

124. The reusable linker arm defined in claim 123, wherein a is 0 and R^a is -OH..

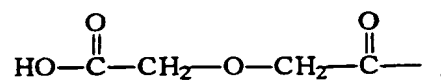
125. The reusable linker arm defined in claim 123, wherein a is 1 and R^a is -NR or -OR.

126. The process defined in claims 123-125, wherein the protecting group is selected from the group comprising acetyl, chloroacetyl, methoxyacetyl, t-butyl phenoxyacetyl, trityl, methoxytrityl, dimethoxytrityl (DMT), dialkylphosphite, pivalyl, isobutyloxycarbonyl, t-butyl dimethylsilyl, phenoxyacetal, 9-phenylxanthen-9-yl (pixyl), tetrahydropyranyl, methoxytetrahydropyranyl, methoxymethyl, benzylloxymethyl, methoxyethoxymethyl, methylthiomethyl, dialkylphosphate, levuliny, dimethylphenylsilyl, trimethylsilyl, isopropyl dimethylsilyl, diisopropyl methylsilyl, diethylisopropylsilyl, triisopropylsilyl, benzoyl, pivaloyl, trifluoroacetyl, allyl, benzyl, o-nitrobenzyl, o-hydroxystyryl dimethylsilyl, 2-oxo-1,2-diphenylethyl, allyloxycarbonyl, monomethoxymethyl, nitroveratryloxycarbonyl, dimethoxybenzoin, dimethoxybenzoin carbonate, methyl nitropiperonyl carbonate, fluorenylmethoxycarbonyl, 2-phenylsulfonyl-ethoxycarbonyl, fluorophenyl-methoxypiperidiny and mixtures thereof.

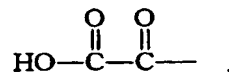
127. The process defined in claims 106-126, wherein Z has the following formula:



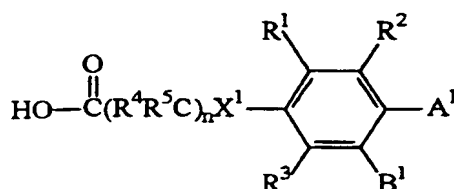
128. The process defined in claims 106-126, wherein Z has the following formula:



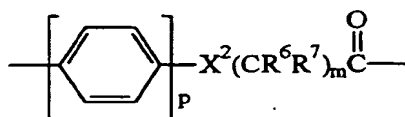
129. The process defined in claims 106-126, wherein Z has the following formula:



130. The process defined in claims 106-126, wherein Z has the following formula:



wherein: R^1 , R^2 and R^3 are the same or different and are selected from the group consisting of hydrogen, halide, a substituted or unsubstituted $\text{C}_1\text{-C}_{20}$ alkyl group, a substituted or unsubstituted $\text{C}_3\text{-C}_{30}$ aryl group and a substituted or unsubstituted $\text{C}_3\text{-C}_{40}$ alkylaryl group; R^4 and R^5 are the same or different and are selected from the group consisting of hydrogen, a substituted or unsubstituted $\text{C}_1\text{-C}_{20}$ alkyl group, a substituted or unsubstituted $\text{C}_3\text{-C}_{30}$ aryl group and a substituted or unsubstituted $\text{C}_3\text{-C}_{40}$ alkylaryl group; X^1 is selected from the group consisting of $-\text{O}-$, $-\text{S}-$, $-\text{C}(\text{O})-$, $-\text{S}(\text{O})_2-$ and $-\text{N}(\text{R})-$; R is selected from the group comprising hydrogen, a substituted or unsubstituted $\text{C}_1\text{-C}_{20}$ alkyl group, a substituted or unsubstituted $\text{C}_3\text{-C}_{30}$ aryl group and a substituted or unsubstituted $\text{C}_3\text{-C}_{40}$ alkylaryl group; n is 0, 1 or 2; and one of A^1 and B^1 is selected from the group consisting of hydrogen, halide, a substituted or unsubstituted $\text{C}_1\text{-C}_{20}$ alkyl group, a substituted or unsubstituted $\text{C}_3\text{-C}_{30}$ aryl group and a substituted or unsubstituted $\text{C}_3\text{-C}_{40}$ alkylaryl group, and the other of A^1 and B^1 has the formula:



wherein p is 0 or 1, X^2 is selected from the group consisting of $-\text{O}-$, $-\text{S}-$, $-\text{C}(\text{O})-$, $-\text{S}(\text{O})_2-$ and $-\text{N}(\text{R})-$, R is selected from the group comprising hydrogen, a substituted or unsubstituted $\text{C}_1\text{-C}_{20}$ alkyl group, a substituted or unsubstituted $\text{C}_3\text{-C}_{30}$ aryl group and a

substituted or unsubstituted C₃-C₄₀ alkylaryl group, R⁶ and R⁷ are the same or different and are selected from the group comprising hydrogen, a substituted or unsubstituted C₁-C₂₀ alkyl group, a substituted or unsubstituted C₃-C₂₀ aryl group and a substituted or unsubstituted C₃-C₄₀ alkylaryl group, and m is 0, 1 or 2.

131. The process defined in claim 130, wherein p is 0.

132. The process defined in claims 130-131, wherein B¹ is selected from the group consisting of hydrogen, halide, a substituted or unsubstituted C₁-C₂₀ alkyl group, a substituted or unsubstituted C₃-C₂₀ aryl group and a substituted or unsubstituted C₃-C₄₀ alkylaryl group.

133. The process defined in claims 130-132, wherein each of R⁴, R⁵, R⁶ and R⁷ is hydrogen.

134. The process defined in claims 130-133, wherein each of m and n are 1.

135. The process defined in claims 130-134, wherein each of R¹, R² and R³ is hydrogen.

136. The process defined in claims 130-135, wherein X¹ and X² are both -O-.

137. The process defined in claims 106-136, wherein SUPPORT is an inorganic substance.

138. The process defined in claim 137, wherein the inorganic substance is selected from the group consisting of silica, glass beads, porous glass, aluminosilicates, borosilicates, metal oxides, clays and mixtures thereof.

139. The process defined in claims 106-136, wherein SUPPORT is an organic substance.

140. The process defined in claim 139, wherein the organic substance is a cross-linked polymer.

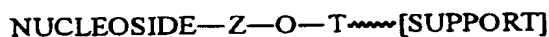
141. The process defined in claims 106-140, wherein the process is conducted in the presence of an activating agent.

142. The process defined in claim 141, wherein the activating agent comprises at least one member selected from the group comprising an acid chloride; an active ester (e.g., nitrophenyl, nitrophenylthio, trichlorophenyl, trifluorophenyl, pentachlorophenyl, pentafluorophenyl, or 3-hydroxy-2,3-dihydro-4-oxo-benzotriazine esters); an active hydroxylamine ester (e.g., N-hydroxyphthalimide or N-hydroxysuccinimide); acid anhydride and mixed anhydride.

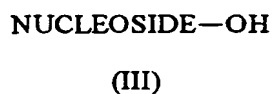
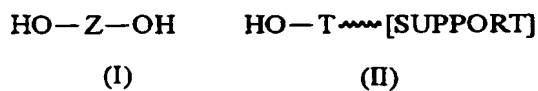
143. The process defined in claim 141, wherein the activating agent comprises at least one member selected from the group comprising arylsulfonyl chlorides (e.g., benzenesulfonyl chloride (BS-Cl), mesitylenesulfonyl chloride (MS-Cl), triisopropylsulfonylchloride (TPS-Cl)); active arylsulfonyl esters (e.g., imidazole, triazole, nitrotriazole, or tetrazole esters of BS-Cl, MS-Cl or TPS-Cl); 2-ethoxy-1-(ethoxycarbonyl)-1,2-dihydroquinoline (EEDQ); acyl carbonates; 1,1'-(carbonyldioxy)dibenzotriazoles; chlorotrimethylsilane; carbodiimides (e.g., dicyclohexylcarbodiimide (DCC), 1-(3-dimethylaminopropyl)-ethylcarbodiimide (DEC), diisopropylcarbodiimide (DIC)) either alone or in combination with auxillary nucleophiles (e.g., 1-hydroxybenzotriazole (HOBt), 1-hydroxy-7-azabenzotriazole (HOAt), N-hydroxysuccinimide (HOSu), or 3-hydroxy-3,4-dihydro-1,2,3-benzotriazin-4-one (HOObt)) and/or catalysts (e.g., 4-dimethylaminopyridine (DMAP) or N-methylimidazole (NMI)); or uronium salts (e.g., tetramethyluronium chloride (TMU-Cl), 2-(1H-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HBTU), 2-

(1H-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate (TBTU), 2-succinimido-1,1,3,3-tetramethyluronium tetrafluoroborate (TSTU), 2-(3,4-dihydro-4-oxo-1,2,3-benzotriazin-3-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate (TDBTU), 2-(2-oxo-1(2H)-pyridyl-1,1,3,3-tetramethyluronium tetrafluoroborate (TPTU), 2-(5-norbornene-2,3-dicarboximido)-1,1,3,3-tetramethyluronium tetrafluoroborate (TNTU), O-(7-azabenzotriazol-1-yl)-1,3-dimethyl-1,3-dimethylenuronium hexa-fluorophosphate (HAMDU), O-(7-azabenzotriazol-1-yl)-1,3-dimethyl-1,3-tri-methylenuronium hexafluorophosphate (HAMTU), O-(7-azabenzotriazol-1-yl)-1,1,3,3-bis(pentamethylene)uronium hexafluorophosphate (HAPipU), O-(7-azabenzotriazol-1-yl)-1,1,3,3-bis(tetramethylene)uronium hexafluorophosphate (HAPyU), O-(7-azabenzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HATU)) either alone or in combination with auxillary nucleophiles (i.e., 1-hydroxybenzotriazole (HOBt), 1-hydroxy-7-azabenzotriazole (HOAt), N-hydroxysuccinimide (HOSu), or 3-hydroxy-3,4-dihydro-1,2,3-benzotriazin-4-one (HOObt)) and/or catalysts (e.g., 4-dimethylaminopyridine (DMAP) or N-methylimidazole (NMI)) or phosphonium salts (e.g., benzotriazol-1-yl-oxytris(dimethylamino)phosphonium hexafluorophosphate (BOP), benzotriazole-1-yl-oxy-trispyrrolidinophosphonium hexafluorophosphate (PyBOP), 2-(benzotriazol-1-yl)oxy-1,3-dimethylimidazolidinium hexafluorophosphate (BOI), bromo tris(pyrrolidino)phosphonium hexafluorophosphate (PyBroP), 7-azabenzotriazol-1-yloxytris-(dimethylamino)phosphonium hexafluorophosphate (AOP), and 7-azabenzotriazol-1-yloxytris(pyrrolidino)phosphonium hexafluorophosphate (PyAOP)) either alone or in combination with auxillary nucleophiles and/or catalysts.

144. A process for production of a reusable linker arm for oligonucleotide synthesis having the following formula:



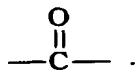
wherein Z is a linker moiety and T is an organic radical, the process comprising the step of reacting together the compounds of Formulae I, II and III:



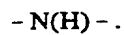
wherein Z and T are as defined above.

145. The process defined in claim 144, wherein T contains at least one carbon.
146. The process defined in claim 144, wherein T is a C₁-C₃₀₀ organic moiety.
147. The process defined in claim 144, wherein T is a C₁-C₂₀₀ organic moiety.
148. The process defined in claim 144, wherein T is a C₁-C₁₀₀ organic moiety.
149. The process defined in claims 144-148, wherein T is a saturated organic moiety.
150. The process defined in claims 144-148, wherein T is an unsaturated organic moiety.
151. The process defined in claims 144-148, wherein T is a C₁-C₃₀₀ organic moiety comprising at least one heteroatom selected from N and O.

152. The process defined in claims 144-151, wherein the organic moiety comprises at least one moiety having the formula:



153. The process defined in claims 144-151, wherein the organic moiety comprises at least one moiety having the formula:



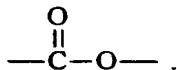
154. The process defined in claims 144-151, wherein the organic moiety comprises at least one moiety having the formula:



155. The process defined in claims 144-151, wherein the organic moiety comprises at least one moiety having the formula:



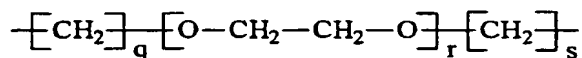
156. The process defined in claims 144-151, wherein organic moiety comprises at least one moiety having the formula:



157. The process defined in claims 144-156, wherein the organic moiety is unsubstituted.

158. The process defined in claims 144-156, wherein the organic moiety is substituted by at least one moiety selected from the group comprising a C₁-C₄₀ alkyl group, a C₃-C₄₀ aryl group, a C₁-C₄₀ alkoxy group, a C₁-C₄₀ ester group, a C₁-C₄₀ hydroxy group, a C₂-C₄₀ acrylate group and a C₃-C₄₀ alkylaryl group.

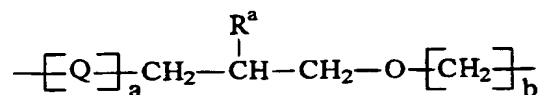
159. The process defined in claims 144-158, wherein T has the formula:



wherein q and s are the same or different and each is an integer having a value of 0-40 and r is an integer having a value of 1-200.

160. The process defined in claim 159, wherein q and s are the same or different and each is an integer having a value of 1-20 and r is an integer having a value of 1-150.

161. The process defined in claim 144-158, wherein T has the formula:



wherein a is 0 or 1, Q is an organic moiety, R^a is selected from -OH, -NH₂, -NR and -OR wherein R is a protecting group and b is an integer having a value of 0-40.

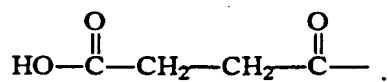
162. The reusable linker arm defined in claim 161, wherein a is 0 and R^a is -OH..

163. The reusable linker arm defined in claim 161, wherein a is 1 and R^a is -NR or -OR.

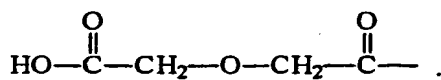
R^a

164. The process defined in claims 161-163, wherein the protecting group is selected from the group comprising acetyl, chloroacetyl, methoxyacetyl, t-butyl phenoxyacetyl, trityl, methoxytrityl, dimethoxytrityl (DMT), dialkylphosphite, pivalyl, isobutyloxycarbonyl, t-butyl dimethylsilyl, phenoxyacetal, 9-phenylxanthene-9-yl (pixyl), tetrahydropyranyl, methoxytetrahydropyranyl, methoxymethyl, benzyloxymethyl, methoxyethoxymethyl, methylthiomethyl, dialkylphosphate, levulinyl, dimethylphenylsilyl, trimethylsilyl, isopropyl dimethylsilyl, diisopropyl methylsilyl, diethylisopropylsilyl, triisopropylsilyl, benzoyl, pivaloyl, trifluoroacetyl, allyl, benzyl, o-nitrobenzyl, o-hydroxystyryl dimethylsilyl, 2-oxo-1,2-diphenylethyl, allyloxycarbonyl, monomethoxymethyl, nitroveratryloxycarbonyl, dimethoxybenzoin, dimethoxybenzoin carbonate, methylnitropiperonyl carbonate, fluorenylmethoxycarbonyl, 2-phenylsulfonyl-ethoxycarbonyl, fluorophenyl-methoxypiperidiny and mixtures thereof.

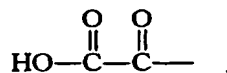
165. The process defined in claims 144-164, wherein Z has the following formula:



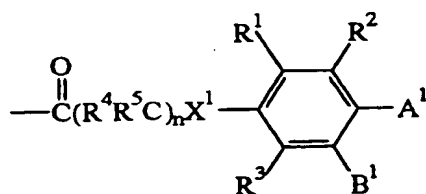
166. The process defined in claims 144-164, wherein Z has the following formula:



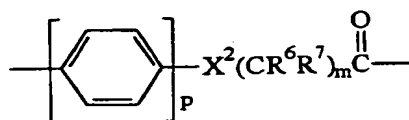
167. The process defined in claims 144-164, wherein Z has the following formula:



168. The process defined in claims 144-164, wherein Z has the following formula:



wherein: R^1 , R^2 and R^3 are the same or different and are selected from the group consisting of hydrogen, halide, a substituted or unsubstituted $\text{C}_1\text{-C}_{20}$ alkyl group, a substituted or unsubstituted $\text{C}_3\text{-C}_{30}$ aryl group and a substituted or unsubstituted $\text{C}_3\text{-C}_{40}$ alkylaryl group; R^4 and R^5 are the same or different and are selected from the group consisting of hydrogen, a substituted or unsubstituted $\text{C}_1\text{-C}_{20}$ alkyl group, a substituted or unsubstituted $\text{C}_3\text{-C}_{30}$ aryl group and a substituted or unsubstituted $\text{C}_3\text{-C}_{40}$ alkylaryl group; X^1 is selected from the group consisting of $-\text{O}-$, $-\text{S}-$, $-\text{C}(\text{O})-$, $-\text{S}(\text{O})_2-$ and $-\text{N}(\text{R})-$; R is selected from the group comprising hydrogen, a substituted or unsubstituted $\text{C}_1\text{-C}_{20}$ alkyl group, a substituted or unsubstituted $\text{C}_3\text{-C}_{30}$ aryl group and a substituted or unsubstituted $\text{C}_3\text{-C}_{40}$ alkylaryl group; n is 0, 1 or 2; and one of A^1 and B^1 is selected from the group consisting of hydrogen, halide, a substituted or unsubstituted $\text{C}_1\text{-C}_{20}$ alkyl group, a substituted or unsubstituted $\text{C}_3\text{-C}_{30}$ aryl group and a substituted or unsubstituted $\text{C}_3\text{-C}_{40}$ alkylaryl group, and the other of A^1 and B^1 has the formula:



wherein p is 0 or 1, X^2 is selected from the group consisting of $-\text{O}-$, $-\text{S}-$, $-\text{C}(\text{O})-$, $-\text{S}(\text{O})_2-$ and $-\text{N}(\text{R})-$, R is selected from the group comprising hydrogen, a substituted or unsubstituted $\text{C}_1\text{-C}_{20}$ alkyl group, a substituted or unsubstituted $\text{C}_3\text{-C}_{30}$ aryl group and a

substituted or unsubstituted C₃-C₄₀ alkylaryl group, R⁶ and R⁷ are the same or different and are selected from the group comprising hydrogen, a substituted or unsubstituted C₁-C₂₀ alkyl group, a substituted or unsubstituted C₃-C₃₀ aryl group and a substituted or unsubstituted C₃-C₄₀ alkylaryl group, and m is 0, 1 or 2.

169. The process defined in claim 168, wherein p is 0.

170. The process defined in claims 168-169, wherein B¹ is selected from the group consisting of hydrogen, halide, a substituted or unsubstituted C₁-C₂₀ alkyl group, a substituted or unsubstituted C₃-C₃₀ aryl group and a substituted or unsubstituted C₃-C₄₀ alkylaryl group.

171. The process defined in claims 168-170, wherein each of R⁴, R⁵, R⁶ and R⁷ is hydrogen.

172. The process defined in claims 168-171, wherein each of m and n are 1.

173. The process defined in claims 168-172, wherein each of R¹, R² and R³ is hydrogen.

174. The process defined in claims 168-172, wherein X¹ and X² are both -O-.

175. The process defined in claims 144-174, wherein SUPPORT is an inorganic substance.

176. The process defined in claim 175, wherein the inorganic substance is selected from the group consisting of silica, glass beads, porous glass, aluminosilicates, borosilicates, metal oxides, clays and mixtures thereof.

177. The process defined in claims 144-174, wherein SUPPORT is an organic substance.

178. The process defined in claim 177, wherein the organic substance is a cross-linked polymer.

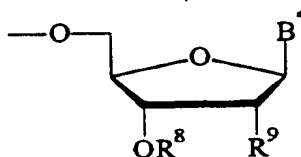
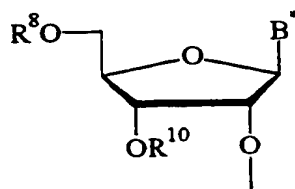
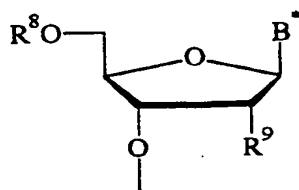
179. The process defined in claims 144-178, wherein the process is conducted in the presence of an activating agent.

180. The process defined in claim 179, wherein the activating agent comprises at least one member selected from the group comprising an acid chloride; an active ester (e.g., nitrophenyl, nitrophenylthio, trichlorophenyl, trifluorophenyl, pentachlorophenyl, pentafluorophenyl, or 3-hydroxy-2,3-dihydro-4-oxo-benzotriazine esters); an active hydroxylamine ester (e.g., N-hydroxyphthalimide or N-hydroxysuccinimide); acid anhydride and mixed anhydride.

181. The process defined in claim 179, wherein the activating agent comprises at least one member selected from the group comprising arylsulfonyl chlorides (e.g., benzenesulfonyl chloride (BS-Cl), mesitylenesulfonyl chloride (MS-Cl), triisopropylsulfonylchloride (TPS-Cl)); active arylsulfonyl esters (e.g., imidazole, triazole, nitrotriazole, or tetrazole esters of BS-Cl, MS-Cl or TPS-Cl); 2-ethoxy-1-(ethoxycarbonyl)-1,2-dihydroquinoline (EEDQ); acyl carbonates; 1,1'-(carbonyldioxy)dibenzotriazoles; chlorotrimethylsilane; carbodiimides (e.g., dicyclohexylcarbodiimide (DCC), 1-(3-dimethylaminopropyl)-ethylcarbodiimide (DEC), diisopropylcarbodiimide (DIC)) either alone or in combination with auxiliary nucleophiles (e.g., 1-hydroxybenzotriazole (HOBt), 1-hydroxy-7-azabenzotriazole (HOAt), N-hydroxysuccinimide (HOSu), or 3-hydroxy-3,4-dihydro-1,2,3-benzotriazin-4-one (HOObt)) and/or catalysts (e.g., 4-dimethylaminopyridine (DMAP) or N-methylimidazole (NMI)); or uronium salts (e.g., tetramethyluronium chloride (TMU-Cl), 2-(1H-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HBTU), 2-

Figure 1. The effect of the concentration of the *Agrobacterium* suspension on the transformation efficiency of *Agrobacterium* strains. The *Agrobacterium* strains were grown in the medium containing 100 mg/l of tetracycline. The cell concentration of the strains was adjusted to 10⁸ cells/ml. The cell suspension was mixed with the plant tissue and the transformation efficiency was determined. The results are the mean of three independent experiments. Error bars represent standard deviation.

-81-



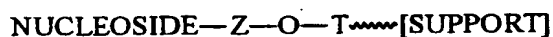
wherein R^8 and R^{10} are the same or different and are hydrogen or a protecting group, R^9 is hydrogen or $-OR^{11}$ wherein R^{11} is hydrogen or a protecting group, and B^+ is a nucleic acid base.

183. The process defined in claims 144-182, wherein the compounds of Formulae I and II are initially reacted to form a conjugate which is reacted with the compound of Formula III.

184. The process defined in claims 144-182, wherein compounds of Formulae I and III are initially reacted to form a conjugate which is reacted with the compound of Formula II.

185. A process for producing an oligonucleotide having a desired sequence comprising the steps of:

- (i) reacting a linker arm having the formula:



wherein Z is a linker moiety and T is an organic radical, with at least one oligonucleoside base until an oligonucleotide having the desired sequence is produce;

- (ii) cleaving the oligonucleotide having the desired sequence to produce a free oligonucleotide have the desired sequence; and a used linker arm; and
- (iii) recycling the used linker arm to Step (i).

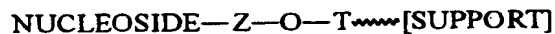
186. The process defined in claim 185, wherein the used linker arm produced in Step (ii) has the formula:

T



wherein Z is a linker moiety and T is an organic radical.

187. The process defined in claims 185-186, wherein Step (iii) comprises the step of converting the used linker arm to a linker arm having the formula: -



wherein Z is a linker moiety and T is an organic radical.

Table 1. *Salmonella* serotypes and phage types isolated from the faeces of the 1000 broiler chickens

$$\text{Z-O-T} \sim [\text{SUPPORT}]$$

-84-

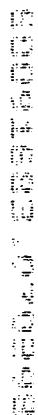
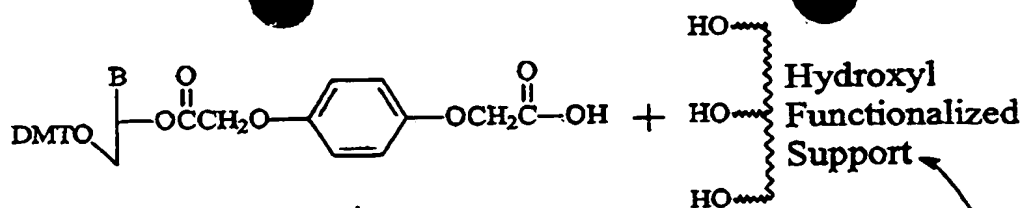
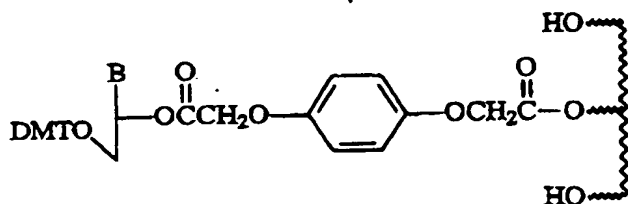


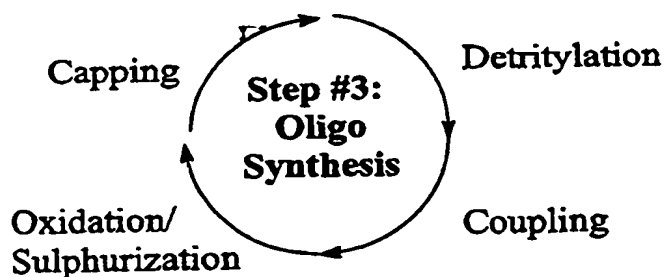
FIGURE 1
(PRIOR ART)



Step #1: Nucleoside Derivatization
 HBTU/DMAP/DIEA
 MeCN, 10 min, R.T.

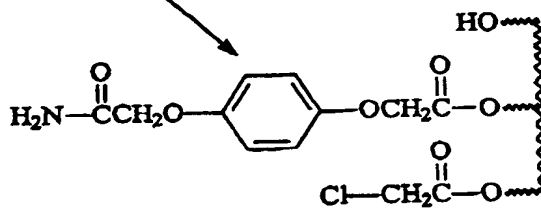


Step #2: Capping
 $(\text{Cl-Ac})_2\text{O/NMI/Lutidine}$
 THF, 5 min



Step #4: Cleavage
 NH_4OH , 5-15 min

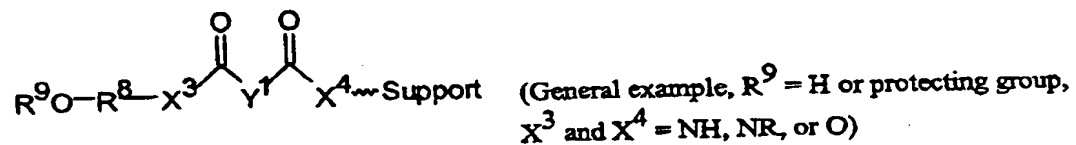
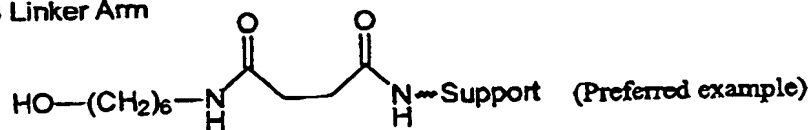
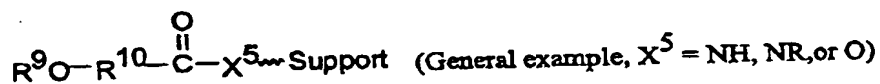
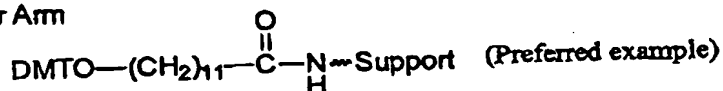
Deprotection
 Purification
 Oligonucleotide Product



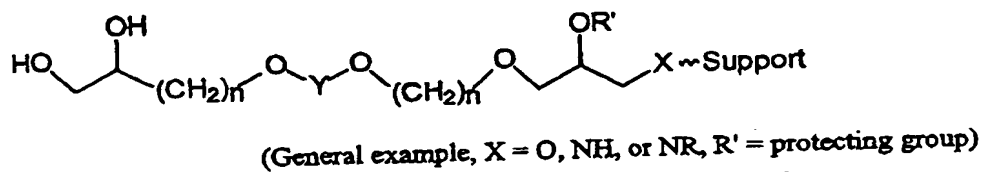
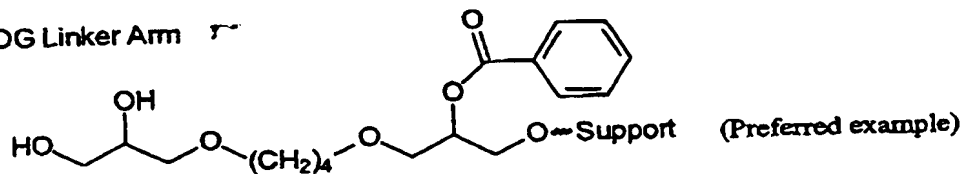
Step #5: Support Regeneration
 $0.05 \text{ M K}_2\text{CO}_3$
 MeOH, 5 Min

FIGURE 2

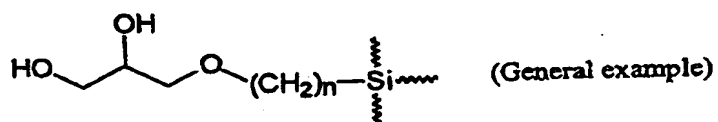
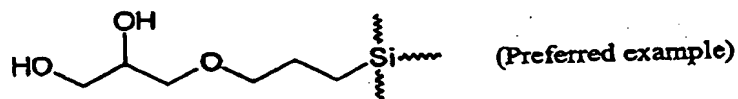
A, OHS Linker Arm

B, C₁₂ Linker Arm

C, BDG Linker Arm



D, GLY Linker Arm (Gly-CPG)

**FIGURE 3**

**This Page is Inserted by IFW Indexing and Scanning
Operations and is not part of the Official Record**

BEST AVAILABLE IMAGES

Defective images within this document are accurate representations of the original documents submitted by the applicant.

Defects in the images include but are not limited to the items checked:

- ☒ **BLACK BORDERS**
- ☐ **IMAGE CUT OFF AT TOP, BOTTOM OR SIDES**
- ☐ **FADED TEXT OR DRAWING**
- ☐ **BLURRED OR ILLEGIBLE TEXT OR DRAWING**
- ☐ **SKEWED/SLANTED IMAGES**
- ☐ **COLOR OR BLACK AND WHITE PHOTOGRAPHS**
- ☐ **GRAY SCALE DOCUMENTS**
- ☐ **LINES OR MARKS ON ORIGINAL DOCUMENT**
- ☐ **REFERENCE(S) OR EXHIBIT(S) SUBMITTED ARE POOR QUALITY**
- ☐ **OTHER:** _____

IMAGES ARE BEST AVAILABLE COPY.

As rescanning these documents will not correct the image problems checked, please do not report these problems to the IFW Image Problem Mailbox.

THIS PAGE BLANK (USPTO)